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EFFECT OF CORTISONE ON THE EXCRETION OF 17-KETOSTEROIDS IN ADRENAL TUMOR*

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THE clinical use of cortisone for the suppression of adrenal hyperactivity in patients with the adrenogenital syndrome has been applied successfully by Wilkins *et al.* (1, 2), Bartter *et al.* (3) and Sheppard and Clausen (4). In all their cases, hyperfunction was associated with adrenal hyperplasia and the administration of cortisone resulted in a marked decrease in the output of urinary 17-ketosteroids.

The question as to whether cortisone would suppress the activity of an adrenal tumor has been a subject of debate. In this report, 4 cases of adrenal hyperfunction treated with cortisone are presented; in 3 of them adrenal tumors were present. The following is a history of these cases:

Case 1—D. M.[1]

The patient, a 3½-year-old white girl, was admitted to the Children's Memorial Hospital on November 1, 1951, with signs of virilization. She was the youngest of 8 siblings, all in good health. The mother suffered from asthma, and the maternal grandmother from diabetes. There was no consanguinity. She had been a normal child until five months previously, when the mother noted that the child's voice was becoming deeper. Pubic hair and facial acne appeared, and the labia majora enlarged and became

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[1] Case 1 was referred to the Children's Memorial Hospital of Montreal by Dr. Ian Gourley of Cornwall, Ontario, with the diagnosis of virilizing adenoma or carcinoma of the left adrenal cortex.

dusky red in color. Enlargement of the abdomen and abdominal pain following meals was complained of for two months preceding admission, and hirsutism of the back, thighs and upper lip had appeared within the last month. Frequent frontal headaches were present during the 5-month period.

On examination the patient was a well nourished little girl of the expected height for her age, weighing 34 pounds. The facial expression was mature for her age and her manner composed. The voice was deep and husky. Muscular development was good. The skin was thick and swarthy, and there was an acneiform eruption over the face. Twenty teeth were present. The scalp hair was thick and black, as was the pubic hair. Numerous black hairs were present over the legs and back, and there was a well marked moustache. The thyroid was not enlarged. Heart and lungs were normal, and the blood pressure 130/90. The abdomen was prominent. On palpation a large firm mass was felt in the left flank, which was non-tender. The clitoris was hypertrophied and measured 2.5 cm. in length. The skin of the labia majora was reddened and thickened.

The white blood count was 4,900 with 2,158 neutrophils and 2,156 lymphocytes; 49 basophils were seen on the smear. The eosinophils numbered 32 per cu.mm. The urine was normal. The concentrations of blood NPN, total proteins, albumin, globulin and cholesterol were within normal limits. The thymol turbidity was 1.8 units and cephalin-cholesterol flocculation negative. The oral glucose time curve was as follows: fasting 117 mg. per cent, ½ hour 119 mg., 1 hour 131 mg., 2 hours 121 mg., and 2½ hours, 92 mg. per cent. Roentgenograms showed a normal skull and chest. The bone age was approxi-

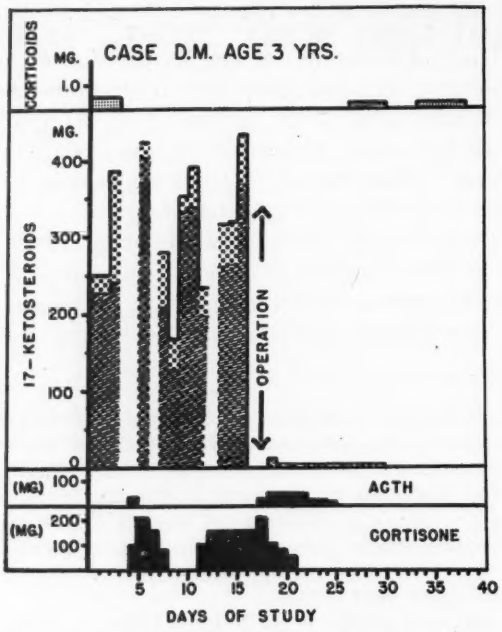

FIG. 1. *Case 1*—Excretion of 17-ketosteroids and corticoids following administration of cortisone and ACTH in a case of adrenal tumor. The darker areas shown on the excretion curve of 17-ketosteroids represent the β-hydroxy fraction.

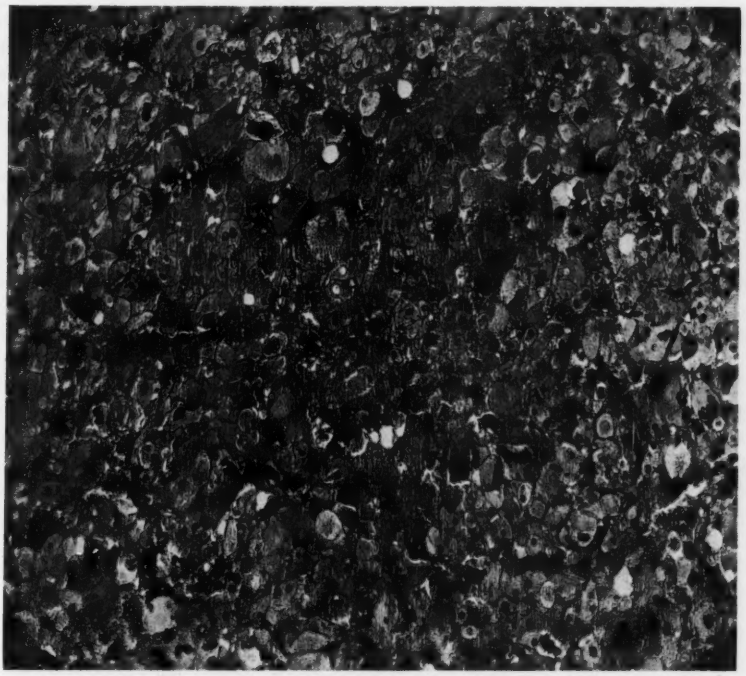

FIG. 2. *Case 1*—Photomicrograph of section from left adrenal. Note the very atypical cells with large bizarre nuclei, and considerable variation in staining intensity. (×100, H & E.)

mately 5 years, by x-ray examination. Films of the abdomen following the injection of Diodrast demonstrated downward displacement of the left kidney by a large soft tissue mass 7–8 cm. in diameter. Two attempts to outline the adrenals by perirenal oxygen insufflation were unsuccessful. An electroencephalogram showed "frequent bursts of paroxysmal medium to high voltage 4 per second slow waves synchronously from both hemispheres." The electrocardiogram was normal. On psychometric testing (revised Stanford-Binet) the mental age was 3 $\frac{5}{12}$ years and the intelligence quotient 111.

The results of fundoscopic examination were negative, apart from slight narrowing of the retinal arterioles. Eosinophils were counted preceding and following the intramuscular injection of 25 mg. of ACTH (Armour, lot 30902). The control eosinophil count was 22 per cu.mm.; at three hours following injection, 44 per cu.mm.; and at five hours, 6 per cu.mm.

The urinary glucocorticoids (bioassay) were 120 μg. per twenty-four hours, and the formaldehydogenic steroids, 0.69 mg. per twenty-four hours. The 17-ketosteroid excretion varied between 246 and 438 mg. per twenty-four hours. Almost all of the ketosteroids excreted were in the β-hydroxy form.

After preliminary investigation the child was given oral cortisone for a number of days preceding operation, in amounts ranging from 75 to 200 mg., as shown on the chart (Fig. 1). On the day of operation and postoperatively, cortisone was injected intramuscularly. At operation the left lumbar approach was used and a large, soft, encapsu-

lated adrenal tumor was visualized and removed. The postoperative course was uneventful, the child being maintained on small doses of cortisone for three days and on ACTH for one week. On the day after operation 17-ketosteroids were 9.7 mg. per twenty-four hours, and 3 days later 1.0 mg. Thereafter they remained below 1 mg. per twenty-four hours. The glucocorticoids and formaldehydogenic steroids were within normal limits when assayed two weeks after removal of the tumor. There was a decline in the creatinine excretion postoperatively. The child was discharged three weeks after operation, to be followed at regular intervals. Two months later the 17-ketosteroids were 0.3 mg. per twenty-four hours.

Pathologic report (*Fig. 2*): The left adrenal mass measured 9.7×8.0×5.0 cm. and weighed 265 Gm. The capsule was grossly intact. The cut surface presented a homogeneous, undifferentiated encephaloid appearance throughout. Microscopically, the tumor cells bore little or no resemblance to the normal adrenal cells, but they did resemble the cells of the fetal cortex to some extent. They varied somewhat in size but were mainly very large, closely packed and rounded or polygonal in shape. There was a large amount of cytoplasm, which was predominantly dense and acidophilic. Other cells were faintly acidophilic and finely granular, and a few were finely vacuolated. The nuclei presented a striking appearance and ranged from small, deeply staining but vesicular nuclei up to huge darkly staining, coarsely granular bizarre-shaped forms. Occasional binucleated and multinucleated cells were present. Large nucleoli were prominent. Mitotic figures were scanty.

Diagnosis: Adenoma of left adrenal, probably malignant.

Case 2—F. R.

A white female child of 5 years was admitted to the Children's Memorial Hospital on September 13, 1951, with signs of precocious sexual development. The child's birth, infancy and early development had not been abnormal. At about the age of 3 years it was noticed that her voice was deepening and becoming more masculine. Pubic hair and scattered long hairs on the thighs made their appearance, and the clitoris began to enlarge. From that time, progressive masculinization occurred. The muscles became strong and heavy, the shoulders broadened and axillary hair appeared.

On examination the child appeared older than 5 years. The height was 49¾ inches and the weight 62½ pounds. The muscles were firm and very well developed. Muscular strength was unusual for her age. The skin was thick, and acne was present on the face and back. The voice was deeper and more husky than that of the average young boy. Nutrition and color were excellent. There was a thick growth of black scalp hair and of pubic and axillary hair. A well marked moustache was present. The thyroid was not enlarged, but the thyroid cartilage was prominent. The breasts and areolae showed no sign of development. The clitoris measured 3 cm. in length and the labia majora were enlarged. The blood pressure was 98/60. On physical examination the chest and abdomen were normal.

The total leucocytes numbered 14,100, neutrophils 5,500, and lymphocytes 7,400. The hemoglobin was 15.1 Gm. per cent and the total eosinophils were 500 per cu. mm. The urine was normal. The level of plasma CO_2 was 21.6 mEq./L., of plasma chlorides 105 mEq./L., and of blood NPN 24.8 mg. per cent. An oral glucose tolerance curve gave the following readings: fasting 53 mg. per cent, ½ hour 76 mg., 1 hour 90 mg., 2 hours 77 mg., and 2½ hours 76 mg. per cent. The bone age was advanced to between 11 and 15 years. The skull was not abnormal by x-ray examination. An intravenous pyelogram showed no displacement of either kidney, but a soft tissue shadow approximately 4 cm.

in diameter was demonstrable immediately above the upper pole of the right kidney. The urinary 17-ketosteroids varied between 70 mg. and 95 mg. per twenty-four hours. The urinary corticoids were 40 μg. per twenty-four hours by bioassay, and 0.86 mg. per twenty-four hours by the chemical method. Urinary estrogen assays gave negative results at 40 m.u. per twenty-four hours.

After preliminary study the child was started on a course of oral cortisone (Fig. 3). She received 150 mg. daily for thirteen days, 125 mg. for one day, then 100 mg., and 25

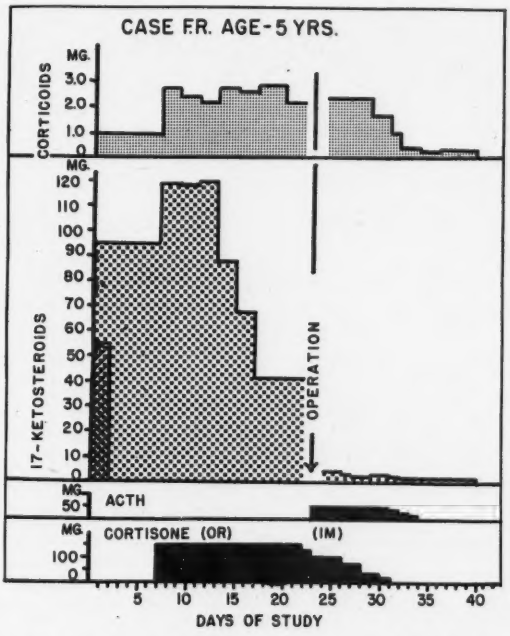

FIG. 3. *Case 2*—Excretion of 17-ketosteroids and corticoids following the administration of cortisone and ACTH in a case of adrenal tumor. Dark column represents the β-hydroxy ketosteroids.

mg. On the day prior to operation she received 150 mg. of cortisone intramuscularly. At operation the abdomen was thoroughly explored. The left adrenal was palpated. It was firm and was estimated to be approximately normal in size. The uterus and ovaries were infantile but not otherwise abnormal. A tumor mass was palpated above the upper pole of the right kidney and was removed without difficulty. The tumor involved the whole right adrenal gland; it was soft and well encapsulated, the capsule being intact. The tumor measured 4 cm. in diameter and was semi-solid in consistency; the liver and surrounding organs revealed no evidence of metastasis.

During operation and throughout the postoperative period the condition of the patient was excellent. She received ACTH in decreasing doses for ten days, and cortisone intramuscularly in decreasing doses for eight days, and was also maintained on penicillin. The excretion of 17-ketosteroids dropped immediately to 3.7 mg. and then more gradu-

ally to normal values of about 1 mg. per twenty-four hours. The serum electrolyte levels were normal and the eosinophil count was between 130 and 420. Because of withdrawn behavior, which alternated with periods of uncooperativeness and indifference, an electroencephalogram was obtained two weeks postoperatively. This showed "frequent bursts of paroxysmal bilaterally synchronous high voltage 3/second slow waves." At the time of operation pubic hair and axillary hair were shaved. Both continued to grow following removal of the tumor, the pubic hair more rapidly than the axillary hair. The

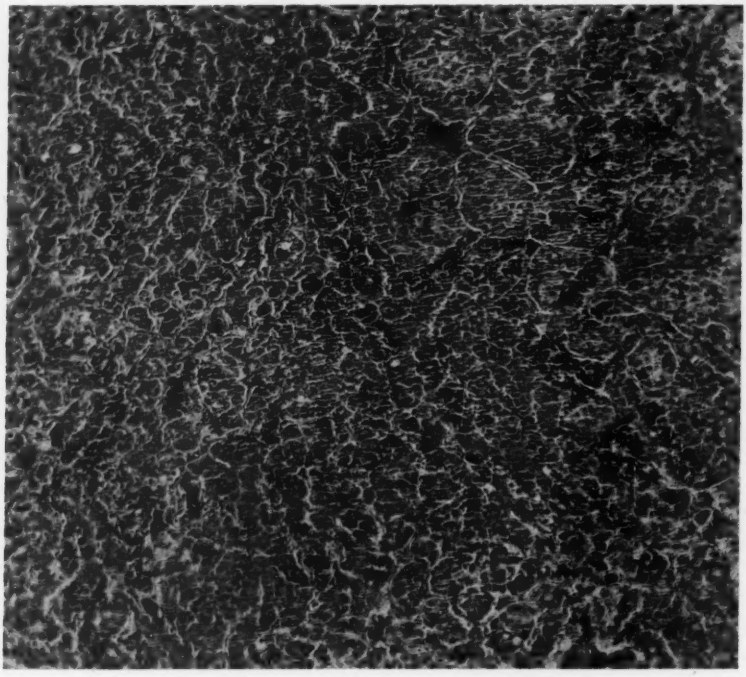

FIG. 4. *Case 2*—Photomicrograph of section from right adrenal gland. Note predominance of small "dark cells" with large pale cells on right. (×100, H & E.)

clitoris appeared smaller six weeks following operation, axillary hair was rather sparse, and the facial expression showed increased femininity. In the seventh postoperative week the patient had scarlet fever. On return from the infectious disease hospital the skin appeared whiter and softer and there was no sign of acne. An electroencephalogram repeated in the third month postoperatively showed definite improvement. The patient was discharged, to be followed at regular intervals. Ten weeks postoperatively the urinary 17-ketosteroids were 0.8 mg. per twenty-four hours.

Pathologic report (*Fig. 4*): The right adrenal gland mass measured 4.3×4.0×3.0 cm. and weighed 25 Gm. The capsule was grossly intact. The cut surface presented a variegated appearance, the greater part of the tumor being of a brownish grey color, with paler areas and yellow flecks throughout. Microscopically, the tumor was well differ-

entiated and bore a striking resemblance to the normal adrenal gland, except for the lack of organized structure. No evidence of capsular invasion was seen. The tumor cells for the most part were arranged in cord-like and trabecular formations, with occasional large sheets of cells. The cells, in the main, consisted of "dark" small cells with a small deeply staining nucleus surrounded by nongranular acidophilic cytoplasm. These resembled the "dark" cells of the normal zona reticulata. No pigment was seen. At the other extreme, there were large cells with a larger vesicular nucleus surrounded by abundant, faintly granular, palely acidophilic cytoplasm. Their cell borders were indistinct and groups of them were vacuolated and foamy. To a certain extent they resembled the cells of the zona fasiculata. Occasional mitotic figures were seen. In addition, there were groups of cells intermediate between and forming transition cells between the two main types.

Diagnosis: Adenoma of the right adrenal gland, possibly benign.

Case 3—R. B.

This patient, aged 29 years, was admitted to the Royal Victoria Hospital on December 15, 1949. She complained of excess hair growth for three years and amenorrhea for two years. Until then she had been well and healthy. Menses began at the age of 15, the interval was twenty-eight days, and the bleeding lasted for three to four days. The menstrual flow gradually become scanty and finally stopped in December 1947. Weight gain was gradual. The face became rounded and acne was severe. Body and facial hair gradually increased, so that at the time of admission she was shaving a heavy beard every second day. There had been temporal recession of the scalp hair, with generalized thinning. She felt there had been some deepening of her voice. She had no other complaints.

Physical examination: pulse 90, B.P. 110/70, weight 148 pounds. The patient was a moderately obese, round faced, ruddy complexioned woman. There was moderate acne over the face and shoulders, with infected sebaceous cysts. The skin was oily, a heavy beard was present, and there was thick coarse hair over the chest and abdomen. The clitoris was enlarged. Rectal examination disclosed a normal sized uterus. No adnexal masses were felt. The remainder of the physical examination showed nothing abnormal.

Laboratory findings: Urine—normal. Blood—Hgb. 119 per cent, and red cells 6,000,000. Glucose tolerance curve—fasting 79 mg. per cent, ½ hour 144 mg., 1 hour 119 mg., 1½ hours 100 mg., 2 hours 93 mg., and 2½ hours 81 mg. per cent. The serum chloride level was 103 mEq./L., potassium 4.8 mEq./L., and sodium 140 mEq./L. Urinary 17-ketosteroids were 65 mg. per twenty-four hours, glucocorticoids 81 μg. per twenty-four hours, and gonadotropin assay was negative at from 6.6 to 105.6 m.u. per twenty-four hours.

Pelvic examination (under anesthesia) showed no abnormality. Hypertrophy of the clitoris was present. Neither ovary was palpated. An intravenous pyelogram gave findings suggestive of a small tumor above the upper pole of the right kidney. X-ray examination of the skull showed the sella turcica to be normal.

In January a laparotomy was performed. No pathologic lesions were found in either ovary. The peritoneum was incised over both kidneys. Both adrenals were felt to be essentially normal. "No evidence of tumor could be felt at this exploration." She made an uneventful recovery from this operation.

In October 1950 she was re-admitted for the purpose of noting the effect of cortisone on adrenal steroid excretion, as well as for further surgery. Since discharge there had been a progression of acne and hair growth. The urinary 17-ketosteroids had increased

to 114–145 mg. per twenty-four hours, and the glucocorticoids were 60 μg. per twenty-four hours. The glucose tolerance curve was within normal limits. Polycythemia continued to be marked. A repeat pyelogram showed a suspicious shadow above the upper pole of the right kidney and on December 4 the right adrenal gland, which contained a tumor, was removed. The left adrenal appeared to be normal in size.

Cortisone and ACTH were administered preoperatively and postoperatively, as in-

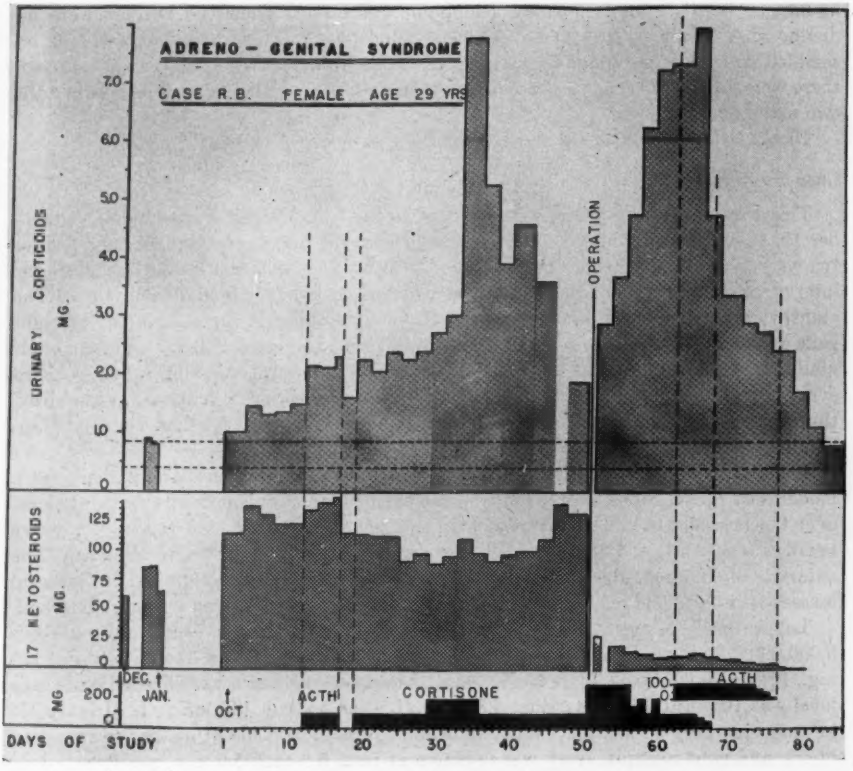

FIG. 5. *Case 3*—Excretion of 17-ketosteroids and corticoids following the administration of cortisone and ACTH in a case of adrenal tumor.

dicated in Figure 5. A thrombophlebitis developed on December 14. On January 1, 1951 menstrual flow began. During the postoperative period the patient remained depressed, weak and hypochondriacal. After ACTH was stopped the 17-ketosteroids were at the low level of 1.3–1.6 mg. per twenty-four hours and the corticoids (chemical determination) and glucocorticoids gradually decreased until the sixth postoperative week. The patient was followed weekly in the endocrine outpatient department. During this period she had almost constant nausea and occasional vomiting. No therapy was given. Finally adrenal function began to improve. Assays performed during the next few months were as follows:

TABLE 1

Postoperative week	17-Ketosteroids (mg./24 hrs.)	Corticoids (formaldehydogenic) (mg./24 hrs.)	Corticoids (biologic) (μg./24 hrs.)
Fourth	1.8	1.70	21
Fifth	1.6	0.78	19
Sixth	1.1	0.27	<10
Ninth	1.2	0.39	30
Twenty-fifth	2.6	0.49	41

Clinical improvement did not occur until the ninth week and was closely correlated with the rise in glucocorticoids. The 17-ketosteroids remained low. Body hair gradually disappeared. At present the patient shaves a light beard once per week. The acne has almost completely disappeared. Menstrual cycles are regular, with a normal flow. The voice remains as it was before, somewhat low. Fourteen months postoperatively, the 17-ketosteroids are 2.8 mg. per twenty-four hours and the corticoids and glucocorticoids within normal range.

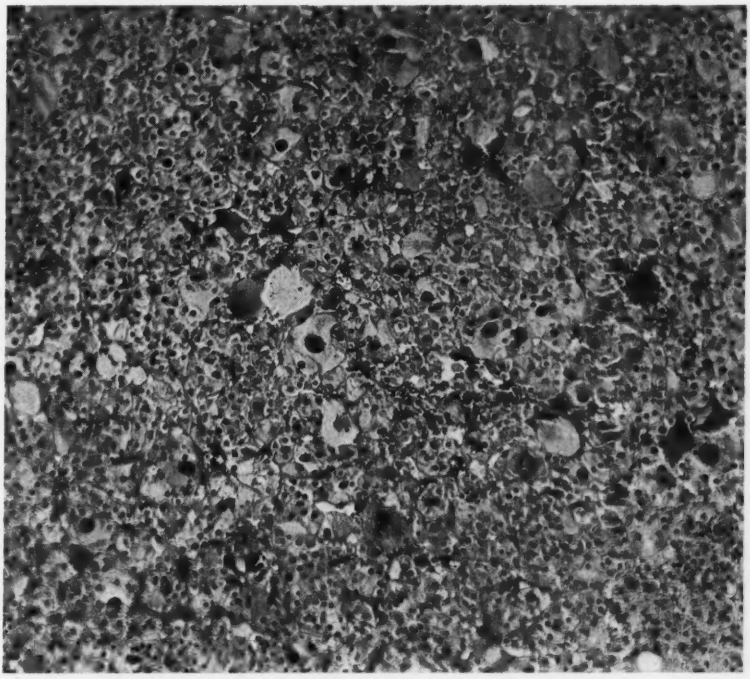

FIG. 6. *Case 3*—Photomicrograph of section from right adrenal gland. The histologic picture is similar to that in Case 1, but the large cells with large bizarre nuclei are less numerous. (×100, H & E.)

Pathologic report (*Fig. 6*): The right adrenal mass measured 4.5 cm. in diameter and weighed 35 Gm. It was pink and encapsulated and the cut surface was soft, fleshy, reddish brown and mottled in appearance. The sections failed to reveal any normal adrenal gland structure. There was a thin outermost fibrous capsule beneath which was seen a thin rim of compressed adrenal cortical cells. These cells had uniform small round dark nuclei and a moderate amount of pale vacuolated cytoplasm. Deep to this zone there was another thicker layer of dense hyaline fibrous connective tissue which encapsulated the large central tumor mass.

The tumor mass was composed of large polyhedral cells which were mostly closely packed but centrally showed a tendency to a cord-like arrangement with intervening thin-walled sinusoids containing blood. These sinusoids were lined by a flat endothelium against which the tumor cells abutted without any intervening stroma. The tumor cells were markedly pleomorphic throughout. The nuclei varied in size and shape and in their chromatin content. The majority were small and round but all gradations were seen, up to tremendous irregular and deeply basophilic ones, many of which contained one or more large red nucleoli. The cytoplasm similarly varied in amount and appearance but in general was abundant, granular and eosinophilic. Vacuolation was noted in some cells, particularly in the peripheral zone. Many of the cells contained intracytoplasmic accumulations of golden brown pigment granules. There were no mitotic figures and no definite capsular invasion or vascular permeation by the tumor cells was noted.

Diagnosis: Adrenal cortical tumor. Distinction between benign and malignant forms of this tumor is not clear cut; in view of marked cellular pleomorphism, it should be regarded as borderline.

Case 4—C. C.

A 15-year-old white female, was admitted to the Royal Victoria Hospital on January 16, 1951. She complained of obesity, which began in the autumn of 1949. Since that time her body weight had increased from 115 to 140 pounds (a gain of 25 pounds). Seven months prior to admission she noticed increased hair growth on the arms, legs, under the chin, and on the chest. The hair had become coarser. Three months before entry, acne began to develop. The skin became greasy, as did the hair. At this time she noted that the interval between the menses, previously 21–28 days, had lengthened to 35–42 days. The flow had become scantier. Until this time she had always been a healthy, active girl.

Physical examination: pulse 86, B.P. 118/80, weight 140 lbs. Mild acne was noted over the face and chest. There was fine, dark hair on cheeks and chin, and over the front of the chest. Hair was increased on the arms and legs. Pubic hair was abundant and of male distribution. Rectal examination was somewhat unsatisfactory. The clitoris was normal in size. No masses were felt. The remainder of the physical examination gave negative results.

Investigation: Urine—normal. Blood—Hgb. 99 per cent, and red cells 4,600,000. Glucose tolerance curve—fasting 109 mg. per cent, ½ hour 201 mg., 1 hour 179 mg., 2 hours 139 mg., and 3 hours 126 mg. per cent. Urinary 17-ketosteroids ranged from 20.1 to 26.9 mg. per twenty-four hours. Urinary glucocorticoids were 81 μg. per twenty-four hours. X-ray examination of the sella turcica showed no abnormality. Bone age was compatible with chronologic age. The patient was asked to return at the end of the school year for more exhaustive investigation. This she did in June, 1951. There had been considerable progression of the masculinizing syndrome since the previous admission. The hair had spread over the cheeks, back, and inner thighs, and had become coarser. The last menstrual period was in January, 1951. Purplish striae had developed over the hips and inner thighs. The blood pressure was 120/80. Hemograms were es-

sentially normal, with red cells 3,100,000, and Hgb. 101 per cent; the direct eosinophil count was 19.

Special investigations were carried out as follows: glucose tolerance curve—fasting 105 mg. per cent, ½ hour 213 mg., 1 hour 238 mg., 1½ hours 174 mg., 2 hours 130 mg., 2½ hours 34 mg., and 3 hours 63 mg. per cent. The concentration of blood NPN was 29.4 mg. per cent, serum sodium 143 mEq./L., serum potassium 4.3 mEq./L., serum calcium 11.0 mg. per cent, and serum cholesterol 268 mg. per cent. Urologic examination—a cystoscopy showed nothing abnormal. A pyelogram gave normal findings, with a suggestion of a soft tissue mass in left suprarenal area. X-ray studies showed a) "There is a mild degree of osteoporosis present in the lumbar vertebrae," and b) an administered Seidlitz powder showed "no evidence of any tumor above upper pole of left kidney."

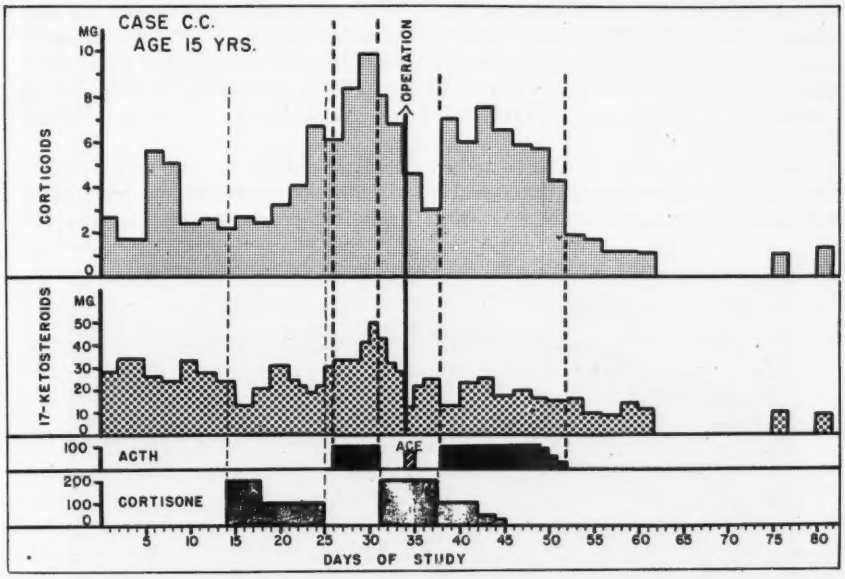

FIG. 7. *Case 4*—Excretion of 17-ketosteroids and corticoids following the administration of cortisone and ACTH in a case of unilateral hyperplasia of the adrenal.

Urinary estrogen assays were positive at 120 m.u. per twenty-four hours, 17-ketosteroids ranged from 24.5 to 33.8 mg., formaldehydogenic steroids from 1.67 to 5.54 mg., and glucocorticoids from 58 to 121 μg. per twenty-four hours.

On June 26, the fourteenth day of study, cortisone (200 mg.) was administered orally for four days and then 100 mg. each day for seven days (Fig. 7). ACTH, 100 mg. daily, was then administered for five days from July 8 through July 12. Exploratory operation was performed on July 16. The right gland was exposed and visualized in its entirety. The size was normal and there was no evidence of carcinoma or adenoma. The left gland proved to be at least twice the size of the right and a questionable cystic space was opened in its central portion. There was no definite adenoma or other tumor present. The entire gland was removed.

On the day of operation 25 cc. of adrenocortical extract (aqueous) was given each time at 11:00 A.M., 2:00 P.M. and 8:00 P.M. Cortisone was administered daily in amounts

shown on the chart. On the fourth postoperative day ACTH therapy was started and continued for fourteen days. The dose of cortisone was gradually reduced and was discontinued after the eleventh day postoperatively.

Potassium chloride was administered (1 Gm., 4 × day) because the serum potassium level following operation was 2.69 mEq./L. The serum potassium level remained within normal limits for the remainder of her hospital stay. On discharge the concentration of serum potassium was 3.96 mEq./L., sodium 144 mEq./L., and NPN 28.4 mg. per cent.

Postoperative levels of urinary 17-ketosteroids and corticoids are shown in Figure 7. The glucose tolerance curve twenty-three days postoperatively was as follows: fasting 66 mg. per cent, ½ hour 132 mg., 1 hour 113 mg., 1½ hours 151 mg., 2 hours 105 mg., and 2½ hours 81 mg. per cent.

The patient left the hospital, well. There has been a gradual improvement clinically. The hair has become of a finer texture. The acne is not as severe. The obesity has been reduced. She has had two normal menstrual periods—December, 1951 and January, 1952. Seven weeks postoperatively the 17-ketosteroids were 10.6 mg. per twenty-four hours. Assays carried out seven months postoperatively showed the excretion of 17-ketosteroids to be 11.6 mg. per twenty-four hours, and that of the corticoids, 0.884 mg. per twenty-four hours.

Pathologic report (*Fig. 8*): The left adrenal mass measured 8×4 cm. and weighed 10 Gm. The cut surface was brownish yellow and friable, the outer surface was greyish pink in color. Several sections of the cortex of the gland revealed a normal architecture.

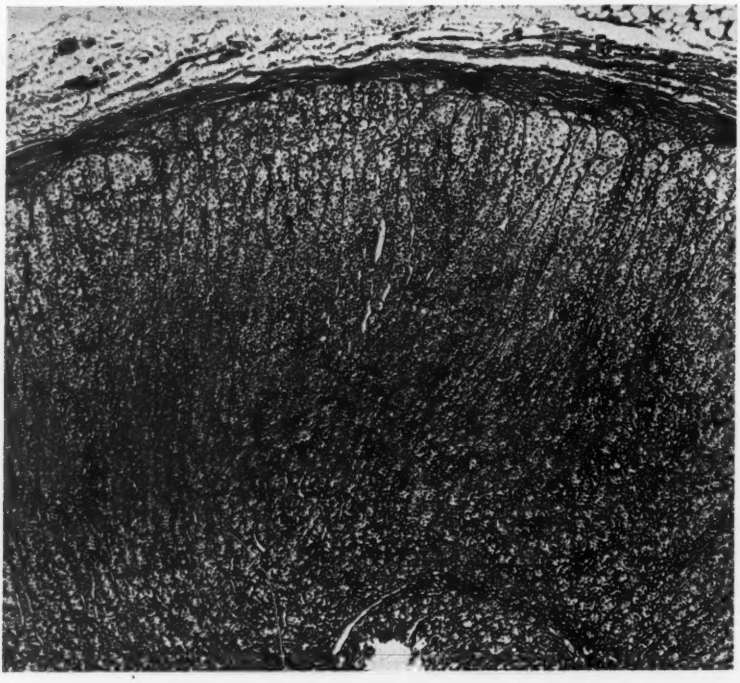

FIG. 8. *Case 4*—Photomicrograph of full width of one side of left adrenal cortex, showing all zones. (×48, H & E.)

The outer zona glomerulosa was narrow and merged imperceptibly with a well developed zona fasciculata, which occupied approximately half the width of the cortex. It consisted of fairly thick columns of large pale polyhedral cells. These cells had small round uniform nuclei and abundant pale granular eosinophilic and vacuolated cytoplasm with clearly outlined cell borders. The cells of the outer portions of the columns were larger and paler and had a ballooned appearance due to their marked vacuolation. The columns were closely packed and showed collapse of the intervening sinusoids, which were lined by flat dark endothelial cells. The inner portion of the zona fasciculata, in turn, merged with the zona reticularis in a poorly defined fashion. The latter formed slightly less than half of the total width of the cortex. The cells tended to be slightly smaller, darker and less vacuolated than those of the zona fasciculata. Their lipochrome pigment content was minimal. Very few pyknotic nuclei were noted. Mitotic activity was not recognized in any of the three zones. The dense fibrous capsule of the gland appeared of normal thickness and the small blood vessels which it contained were not remarkable. There was some hemorrhagic extravasation into the small amount of attached adipose tissue.

Diagnosis: Mild degree of hyperplasia of the adrenal cortex with abundance of lipid content.

METHODS

The 17-ketosteroids were measured by the method of Callow *et al.* (5), and a correction factor to eliminate interfering chromogens was applied (6). The dehydroisoandrosterone fraction was determined by the method of Allen *et al.* (7). The corticoids were measured both by the bioassay of Venning *et al.* (8), and as formaldehydogenic steroids by the chemical procedure of Daughaday *et al.* (9).

RESULTS

Effect of cortisone

In these 4 cases of adrenal hyperfunction, in which hirsutism and virilization were the predominating signs in 3 patients (the fourth having hirsutism and amenorrhea only), cortisone therapy had a varying effect upon the excretion of 17-ketosteroids. In one of the cases (Case 2) the 17-ketosteroid excretion decreased from a level of 95 mg. to 40 mg. per twenty-four hours following the administration of 150 mg. of cortisone orally for fifteen days. In this patient, the 17-ketosteroids increased initially during cortisone therapy and suppression did not occur until the ninth day. Unfortunately cortisone was not administered beyond the fifteenth day, so that it is not possible to know whether the 17-ketosteroid level could have been further decreased. Following removal of the tumor and after cessation of all therapy, the 17-ketosteroids remained around the level of 1.2 mg.

In Case 1 (Fig. 1) the 17-ketosteroids were predominantly of the β-hydroxy form, 63 to 93 per cent of the total being excreted as dehydroisoandrosterone. During the first four days of administration of cortisone the 17-ketosteroids increased to 438 mg. and then fell to 167 mg. per

twenty-four hours. At this point, cortisone therapy was discontinued and readministered after a period of three days. During the second period of study larger quantities of 17-ketosteroids were excreted, amounting to 475 mg. per twenty-four hours on the twelfth day. The β-hydroxy fraction remained high and cortisone did not appear to have any inhibiting effect on the excretion of these steroids. The removal of a large tumor resulted in a lowering of the 17-ketosteroid excretion to 0.6 mg. per twenty-four hours.

In a third patient (Case 3), an adult female aged 29 years, the 17-ketosteroid excretion in the control period averaged 125 mg. per twenty-four hours. Following the administration of cortisone, the 17-ketosteroids slowly decreased to 85 mg. per twenty-four hours. After a period of ten days, the decrease was not sustained and on continued administration of this substance the excretion rate of 17-ketosteroids began to rise so that at the end of thirty days it had returned to levels observed in the control period. At operation a tumor of the right adrenal gland was removed. Although the left adrenal appeared to be normal in size, the 17-ketosteroids have remained low in this patient and fourteen months postoperatively are still at a level of 2.8 mg. per twenty-four hours. The corticoids, on the other hand, by both methods of assay, have returned to normal.

A patient with unilateral hyperplasia of the adrenal gland (Case 4) showed clinical features of both the adrenogenital and Cushing's syndromes, such as impaired glucose tolerance and masculinization without clitoral hypertrophy. The urinary corticoids were elevated and the 17-ketosteroids averaged 28 mg. per twenty-four hours during a fourteen-day control period. During cortisone administration there was initial lowering of the 17-ketosteroids to 13 mg. per twenty-four hours, which was later followed by an increase in output. It is of interest to note in this patient that after the removal of the hyperplastic adrenal the 17-ketosteroids did not decrease to the low levels seen in the other 3 cases but remained at a level of approximately 10 mg. per twenty-four hours.

Effect of ACTH

The effect of ACTH on the excretion of 17-ketosteroids and corticoids was studied in 2 patients. The administration of 100 mg. of ACTH (Armour Standard) daily for five days to the patient with unilateral hyperplasia of the adrenal (Case 4) caused a significant rise in the excretion of corticoids. There appeared to be a delayed response, in the 17-ketosteroid excretion. This patient had received cortisone prior to the administration of ACTH and it is possible that the response of the adrenal cortex may have been altered by this treatment.

A similar amount of ACTH administered to the patient with an adrenal

tumor (Case 3) induced relatively small changes in the excretion of 17-ketosteroids. The increase in urinary corticoids was present but was less marked than in Case 4.

DISCUSSION

The mechanism of the action of cortisone in the suppression of adrenal cortical function has been attributed by Sayers and Sayers (14) to its inhibiting effect on the release of adrenocorticotropin and not to a direct effect of cortisone on the adrenal gland itself. In the hypophysectomized rat maintained on ACTH, Lewis *et al.* (15) found that cortisone does not decrease the size of the adrenal gland, and investigations carried out in man by Bartter *et al.* (3) support this theory.

The observations of Sprague *et al.* (11) that cortisone caused a reduction in the excretion of 17-ketosteroids suggested the possibility of its use for the suppression of adrenal activity in the adrenogenital syndrome. Wilkins *et al.* (2), Bartter *et al.* (3), and Sheppard and Clausen (4) have all reported a lowering of the 17-ketosteroid excretion in this type of case when the overproduction of androgenic substances is associated with adrenal hyperplasia. In none of the 3 patients with adrenal tumor treated with cortisone was it possible to decrease the 17-ketosteroid excretion to normal levels. Cortisone had only a transitory effect in Cases 1 and 3; and in the third, Case 2, the 17-ketosteroids were reduced from 95 to 40 mg. per twenty-four hours. In 2 patients there was an initial temporary increase of 17-ketosteroid excretion and in Case 3, after showing some reduction, the 17-ketosteroid excretion began to increase during continued administration of cortisone. It has been shown (11) that this substance is metabolized in small amounts to 17-ketosteroids and it is possible, although we have no evidence to support this view, that the presence of abnormal adrenal tissue may influence the metabolism of endogenous as well as administered cortisone, resulting in increased 17-ketosteroid production.

It is interesting to note that in Case 4, the patient with unilateral hyperplasia, the 17-ketosteroid excretion was not greatly affected by the daily administration of 200 mg. of cortisone, and that when 100 mg. of ACTH was given for five days there was considerable delay in the rise of 17-ketosteroids, although the urinary corticoids increased in the usual manner. This patient had received cortisone prior to the administration of ACTH and this may have altered the response. Only one adrenal was enlarged and on its removal the 17-ketosteroids returned slowly to the normal range. Administration of ACTH during the postoperative period caused a rise in 17-ketosteroids and corticoids, in spite of the fact that cortisone was being administered at the same time. The possibility that this was a case of early Cushing's syndrome should be kept in mind.

In the patient with an adrenal tumor (Case 3), the responses to ACTH appeared to be diminished as compared with those obtained in normal individuals. Bartter *et al.* (12) found that patients with virilism associated with hyperplasia showed an abnormal response to ACTH, and Eliel and Pearson (13) have reported that ACTH increased all adrenal functions in a patient with Cushing's syndrome, the hyperplasia of the adrenal in this case being the result of pituitary tumor. Lewis and Wilkins (15) observed a normal response to ACTH in a patient with Cushing's syndrome treated with methyltestosterone, and in 2 cases of congenital adrenal hyperplasia with virilism the rise in corticoids following administration of ACTH was less marked.

Further investigations concerning the possibility of using the response to cortisone and ACTH as a means of differentiating between adrenal hyperplasia and adrenal tumor would be of value.

SUMMARY

1. Cortisone and ACTH have been administered to 3 patients with adrenal tumor and 1 patient with unilateral adrenal hyperplasia.

2. The effect of cortisone in the 3 patients with adrenal tumor was a temporary reduction in the urinary excretion of 17-ketosteroids. In 1 case, although there was a significant decrease in the output of 17-ketosteroids over a period of fifteen days, the level was still above normal. In the other 2 cases, after a slight decrease in output, the urinary 17-ketosteroids increased again to high levels. The β-hydroxy fraction was not influenced by cortisone in 1 of these patients.

3. In the one case of unilateral adrenal hyperplasia, cortisone did not significantly decrease the output of 17-ketosteroids.

4. ACTH was administered in a dose of 100 mg. daily for five days to 2 patients, one with adrenal tumor, the other with unilateral hyperplasia of the adrenal. In the first case only small increases in the excretion of of corticoids and 17-ketosteroids occurred, whereas in the second case there was a significant increase in the corticoid excretion, the 17-ketosteroids showing a delayed response.

Addendum

Since completion of these investigations, a paper by Gardner and Migeon (16) has been published on the effect of cortisone on the excretion of 17-ketosteroids in 2 cases of adrenal hyperplasia and 2 cases of adrenal tumor. Administration of this adrenal hormone caused a reduction in the excretion of 17-ketosteroids in the patients with adrenal hyperplasia but no effect was noted in those with adrenal tumor.

Acknowledgments

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REFERENCES

1. Wilkins, L.; Lewis, R. A.; Klein, R., and Rosemberg, E.: Suppression of androgen secretion in congenital adrenal hyperplasia, *Am. J. Dis. Child.* **80:** 883, 1950.
2. Wilkins, L.; Lewis, R. A.; Klein, R.; Gardner, L. I.; Crigler, J. F., Jr.; Rosemberg, E., and Migeon, C. J.: Treatment of congenital adrenal hyperplasia with cortisone, *J. Clin. Endocrinol.* **11:** 1, 1951.
3. Bartter, F. C.; Albright, F.; Forbes, A. P.; Leaf, A.; Dempsey, E., and Carroll, E.: The effect of adrenocorticotropic hormone and cortisone in the adrenogenital syndrome associated with congenital adrenal hyperplasia, *J. Clin. Investigation* **30:** 237, 1951.
4. Sheppard, T. H., and Clausen, S. W.: Case of adrenogenital syndrome treated with cortisone, *Pediatrics* **8:** 805, 1951.
5. Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric determination of substances containing the grouping—CH_2CO—in urine extracts as an indication of androgen content, *Biochem. J.* **32:** 1312, 1938.
6. Engstrom, W. W., and Mason, H. L.: A study of the colorimetric assay of urinary 17-ketosteroids, *Endocrinology* **33:** 229, 1943.
7. Allen, W. M.; Hayward, S. J., and Pinto, A.: A color test for dehydroisoandrosterone and closely related steroids, of use in the diagnosis of adrenocortical tumors, *J. Clin. Endocrinol.* **10:** 54, 1950.
8. Venning, E. H.; Kazmin, V. E., and Bell, J. C.: Biological assay of adrenal corticoids, *Endocrinology* **38:** 79, 1946.
9. Daughaday, W. H.; Jaffe, H., and Williams, R. H.: Chemical assay for "cortin." Determination of formaldehyde liberated on oxidation with periodic acid, *J. Clin. Endocrinol.* **8:** 166, 1948.
10. Lewis, R. A.; Rosemberg, E., and Wilkins, L.: The effects of 17-hydroxy-11-dehydrocorticosterone upon the adrenals of normal and of hypophysectomized rats maintained with adrenocorticotropin, *Endocrinology* **47:** 414, 1950.
11. Sprague, R. G.; Power, M. H.; Mason, H. L.; Albert, A.; Mathieson, D. R.; Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Observations on the physiologic effects of cortisone and ACTH in man, *Arch. Int. Med.* **85:** 199, 1950.
12. Bartter, F. C.; Forbes, A., and Leaf, A.: Congenital adrenal hyperplasia associated with adrenogenital syndrome; an attempt to correct its disordered hormone pattern, *J. Clin. Investigation* **29:** 797, 1950.
13. Eliel, L. P., and Pearson, O. H.: The metabolic effects of adrenocorticotropic hormone (ACTH) in a patient with Cushing's syndrome and acromegaly, *J. Clin. Endocrinol.* **11:** 913, 1951.
14. Sayers, G., and Sayers, M. A.: The pituitary-adrenal system *in* Recent Progress in Hormone Research. New York, Academic Press, Inc., 1948, vol. 2, p. 81.
15. Lewis, R. A., and Wilkins, L.: The effect of adrenocorticotrophic hormone in congenital adrenal hyperplasia with virilism and in Cushing's syndrome treated with methyl testosterone, *J. Clin. Investigation* **28:** 394, 1949.
16. Gardner, L. I., and Migeon, C. J.: Le diagnostic des tumeurs virilisantes du cortex surrénalien: effet de la cortisone sur les stéroïdes urinaires et utilisation d'une méthode colorimétrique pour le dosage de la dehydroisoandrostérone, *Helv. paediat. acta* **6:** 465, 1951.

KLINEFELTER'S SYNDROME: A STUDY OF 5 CASES

HISTOPHYSIOLOGIC BASIS FOR A PATHOGENIC INTERPRETATION*

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KLINEFELTER, Reifenstein and Albright (1) were the first to describe this syndrome of testicular deficiency, which apparently starts at puberty and involves the whole testis. The chief clinical, humoral and histologic characteristics which they noted were: 1) small testes, the size of a chickpea or medium-sized grape, localized in a normally developed scrotum; 2) onset at puberty; 3) azoospermia, or very few spermatozoa present in the semen; 4) an increase in the urinary excretion of hypophyseal gonadotropins; 5) a testicular histologic pattern which shows disappearance of the germ cells (few spermatogonia can be seen), hyalinization of the tubules and morphologic alterations of the Leydig and Sertoli cells; 6) gynecomastia appearing at puberty; 7) usually a reduction in the urinary excretion of neutral 17-ketosteroids, although in some cases it is normal; and 8) usually no clinical manifestations of androgenic deficiency. Later, Heller and Nelson (2) demonstrated in similar cases that gynecomastia is not always present and that the Leydig cells show various abnormalities (Nelson and Heller (3)). In a recent publication, Howard, Sniffen, Simmons and Albright (4) included in this syndrome cases with and without gynecomastia, and accepted the existence of alterations in the Leydig cells.

This work contains the results of the clinical, histologic and hormonal study of 5 cases of Klinefelter's syndrome. Particular attention has been given to the interpretation of the histochemical aspect of the testicular biopsy specimens, in an effort to achieve conclusive physiopathologic knowledge.

METHODS

The following methods were used: *Urinary pituitary gonadotropin assay.* An ultrafilter (Gorbman (5)) was used for extraction. The amount of gonadotropin was estimated

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following the technique recommended by Klinefelter, Albright and Griswold (6). By means of this combined method, de la Balze, Arrillaga and Cárcano (7) found that the rate of excretion of hypophyseal gonadotropins in normal adult males is more than 6.6 and less than 52 mouse units per twenty-four hours. *Urinary 17-ketosteroid assay.* The Zimmermann reaction modified by Callow, Callow and Emmens (8) was used. By this method we found that the range of excretion for normal men is between 8 and 14 mg. per day. *Spermogram.* The routine technique was employed in the examination of seminal fluid. *Testicular biopsy* was carried out according to the technique of Charny and Meranze (9). Local anesthesia of the spermatic cord was obtained with novocaine injections. Specimens were taken from one testis and divided into four sections, each of which was placed in one of the following fixatives: 1) Bouin's fluid for the study of general morphology, glycogen and mucoproteins (mucopolysaccharides and glycoproteins); 2) 10 per cent formol for lipids and silver impregnation; 3) 80 per cent alcohol for alkaline phosphatase; and 4) a special reagent for ascorbic acid. The following techniques were used: 1. For general morphology, the hematoxylin-eosin and the Azan stain. 2. For collagenous and reticular fibers, the silver impregnation of Del Rio Ortega; for elastic fibers, the orcein method. 3. For glycogen, the iodine reaction described by Mancini (10), Bauer's reaction and the Hotchkiss (11) modification of the McManus (12) technique, previously checked by digestion with ptyalin at 37° C. for three hours. 4. For mucopolysaccharides and glycoproteins, the McManus method already mentioned and the photochemical reaction of Etcheverry and Mancini (13), both checked with ptyalin digestion, to eliminate the glycogen; also the metachromatic technique, using thionin and toluidine blue (Lison (14)) and colloidal iron (Hale (15)). In order to find out the type of mucoprotein present in the testes, the digestion with testicular hyaluronidase (Wyeth) in saline solution (20 mg. per cent, pH 6.6) was used for the treatment of the sections prior to the application of the technique mentioned. With this test only two acid mucopolysaccharides (chondroitinsulfuric and hyaluronic acid) are digested. 5. Total lipids by Sudan III and Scarlet red; fatty acids and neutral fats by Nile blue sulphate (Cain (16)); lipids and aldehydes by the plasmal reaction without mercuric chloride; cholesterol esters by the Schultz reaction and dinitrophenylhydrazene (Bennet (17)); and free cholesterol by digitonin and optic polarization. Sections stained by lipid and cholesterol methods, were compared with other sections previously treated with acetone for three hours at room temperature and then stained with the same methods. These groups of tests appear to have some value in determining the existence of steroid-like substance. 6. Alkaline phosphatase, by the Kabat and Fursth (18) modification of the Gomori method. 7. Ascorbic acid, by the Giroud and Leblond technique modified by Deane and Morse (19).

MATERIAL

Five patients (Nos. 1–5) with the diagnosis of Klinefelter's syndrome were studied. Sixteen volunteers ranging from 20 to 70 years of age were used as controls. They were in good health and were normal subjects in respect to testicular morphology, hormonal assays (urinary excretion rate of FSH and neutral 17-ketosteroids) and spermogram (Mancini, Nolasco and de la Balze (20)).

RESULTS

Normal subjects

Urinary neutral 17-ketosteroid and FSH assay (*See* under Methods.)
Testis: histologic pattern

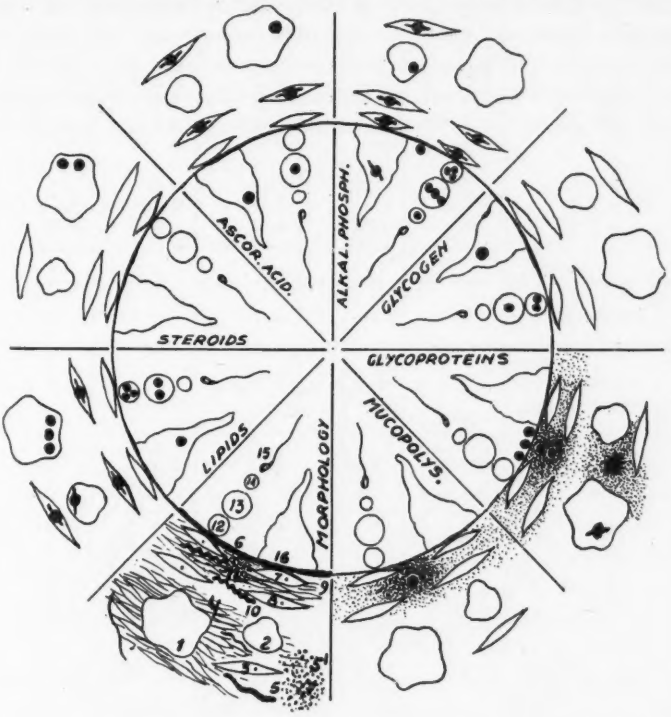
The morphologic and histochemical studies of the characteristics of both the adult men and the Klinefelter syndrome patients were carried out in the following order: 1. Tubules: a) tubule wall; b) Sertoli cells; c) germinal epithelium. 2. Intertubular connective tissue: a) Leydig cells; b) fibroblasts; c) adipose cells; d) collagenous fibers and amorphous substance.

1. *Tubules.* The morphology of germinal epithelium and Sertoli cells is very well known. Concerning the remainder of the testes a brief description of its components follows. The tubule wall is formed of basal membrane and a tunica propria. The basal membrane, comparable to that of other organs, is an apparently homogeneous structure, composed of amorphous substance and a dense net of reticular fibrils and fibrocytes. This membrane is in contact with spermatogonia and Sertoli cells. The tunica propria is composed of an intercellular amorphous substance, reticular fibrils and few elastic fibers. In this part of the wall, fibroblasts are also found. As they approach the inner basal membrane they decrease in size and undergo a condensation of their chromatin content, thus becoming typical fibrocytes.

2. *Intertubular connective tissue.* The Leydig cells are of two different types: a) immature, which are forms of small size, with a thin nuclear membrane and slightly marked nucleolus, slight cytoplasmic basophilia and few perinuclear granules; b) adult forms, which are larger, polyhedral, and surrounded by a well developed reticular net. They are grouped into small nodules with intercellular capillaries. Their cytoplasm is acidophilic, there is a dense accumulation of perinuclear granulation with scattered vacuoles, and occasionally Charcot crystalloids and granules of pigment may be found. The nuclear features—thick nuclear membrane and large nucleolus—complete the typical appearance. The next stage in the evolution of this cell is probably the adipose transformation. The fibroblasts, varying in size and number, and in different stages of development towards the fibrocyte stage, are found scattered between the tubules. The adipose cells, varying in shape and number, seem to originate from the adipose transformation of the Leydig cells. They retain some of the features of the Leydig cells, such as partial or complete vacuolization, but there are no perinuclear granulations. It should be noted that cells with a morphologic appearance suggesting transition from the ordinary fibroblast to immature or mature Leydig cells are often observed in the intertubular connective tissue. In sections tangential to the tube wall, fibroblasts with morphologic features of immaturity are surrounded by an amorphous substance and a fibrillar net. Frequently, in the various intertubular sectors an amorphous substance can be found between the cells of the connective tissue; this substance is acidophilic and of varying density and sometimes it is slightly fibrillar.

In Figure 1 we have tried to locate the substance revealed by the histochemical method used, in the different elements of the testes, according to the foregoing morphologic description. More details can be found in our previous work (Mancini, Nolazco and de la Balze (20)).

Positive histochemical reactions were not always uniform and constant



EXPLANATION OF SCHEMES

FIG. 1. Schematic view of a section of a seminiferous tubule and intertubular connective tissue of normal testis. *Intertubular connective tissue*, with mature Leydig cells [1], immature Leydig cells [2], fibroblasts [3], reticulin fibrils [4], collagen fibers [5], and intercellular amorphous substance [5']. *Tubular wall*, with basal membrane [6] and tunica propria; the latter contains fibrocytes [7], fibroblasts [8], reticulin fibrils [9], elastic fibers [10], and intercellular amorphous substance [11]. *Germinal epithelium*, with spermatogonia [12], spermatocytes [13], spermatids [14], and spermatozoa [15]. *Sertoli cells* [16].

The scheme shows the localization of the various substances studied histochemically in the different structures mentioned. The substances are: lipids, steroids, ascorbic acid, alkaline phosphatase, glycogen, and mucoproteins (glycoproteins, and mucopolysaccharides); each one is represented in a section of the scheme. One section is devoted to morphology and contains the numbers for the different structures. The intensity of the histochemical reactions has been indicated in the following reactions: (•): medium; (••): intense; (•••): very intense. For the sake of clarity cells nuclei and arterioles have been omitted from the scheme.

TABLE 1. SEXUAL CHARACTERISTICS, AND LABORATORY DATA IN 5 CASES OF KLINEFELTER'S SYNDROME

Patient		Phallus	Scrotum	Testis	Epididymis	Voice; thyroid cartilage	Pubic and axillary hair	Beard	Libido; erection; ejaculation; orgasm	Gynecomastia	Prostate	Span & height (cm.)	Urinary FSH (m.u./24 hrs.)	Urinary 17-KS (mg./24 hrs.)	Spermogram
No.	Age (yrs.)														
1	27	=*	Pigm. & corrug.	Small	=	= =	Grew at 15 yrs.; scant.	Scant	= = = =	+	=	S 170; H 169	+6; +104 +6; +13 +52	11 and 12 13 and 14	Azoospermia
2	18	=	Pigm. & corrug.	Small	=	= =	Grew at 16 yrs.; scant.	Scant	= = = =	+	=	S 176; H 170	+6; +52; +104	9.05	Azoospermia
3	19	=	Pigm. & corrug.	Small	=	= =	Grew at 18 yrs.; scant.	Very scant	= = = =	+ + +	=	S 181; H 177	+6; +52; +104	5.08	Azoospermia
4	16	=	Pigm. & corrug.	Small	=	= =	Grew at 14 yrs.; pubic abund.; axillary scant.	Very scant	= = = =	+ +	=	S 185; H 180	+6; +52; +104 +6; +52; +104	4.05	Azoospermia
5	30	=	Pigm. & corrug.	Small	=	= =	Grew at 14 yrs.; normal	Scant	= = = =	+	=	S 181; H 184	+6; +52; −104 −208 −416	9.08	Azoospermia

* (=) indicates normal; (+), moderate; (++), intense; (+++), very intense.

in the whole length of the section. Concerning the intracellular localization, some of the substances studied were found to be displaced, having spread to neighbouring structures. These substances are known to be easily displaced in different tissues by the fixative and other technical agents used. This phenomenon occurs specially with glycogen (Mancini (21)), ascorbic acid (Sosa (22)) and alkaline phosphatase (Leduc and Dempsey (23)). For the description, we have taken into account only the usual cellular disposition. Previous authors have proved that the specificity of the techniques used is questionable, especially with regard to lipids and steroids (Dempsey and Wislocki (24)) and to mucoproteins (Lillie (25), Leblond (26), Mancini (27)).

Klinefelter's syndrome

Physical examination and laboratory findings

Table 1 gives data on secondary sexual characteristics and other features

FIG. 2. Klinefelter's syndrome. Tubular lesion, degree I. (For key to nomenclature *see* Fig. 1.) Increased thickening of basal membrane and tunica propria. More reticular fibers and some collagen fibers begin to appear. Less intense reactions to mucopolysaccharide tests in the tunica propria; reaction to glycoprotein test persists in basal membrane. The fibroblasts of the tubular wall have lost their normal cytochemical characteristics. The Sertoli cells and spermatogonia show slightly positive reactions to histochemical tests.

revealed by physical examination in the 5 patients. The observations are expressed in relation to those on normal adult men. The size of the testes in these cases was about 1.5×0.5×0.5 cm. Gynecomastia was present in 4 cases. Patients 2, 3, and 4 presented eunuchoid proportions. Table 1 also shows the FSH excretion; only the levels indicated in the table were assayed. Patients without eunuchoid proportions revealed the highest value for urinary 17-ketosteroids.

Testes: histologic pattern

The histologic findings, when compared to those of normal testes, confirm the existence of marked changes in the structure and histochemical reactions of tubules and intertubular connective tissue. The changes to be described in this section apply to the 5 cases as a whole, although the degree of change varied from patient to patient. Later we shall show the extent to which the lesion was predominant in each of the patients.

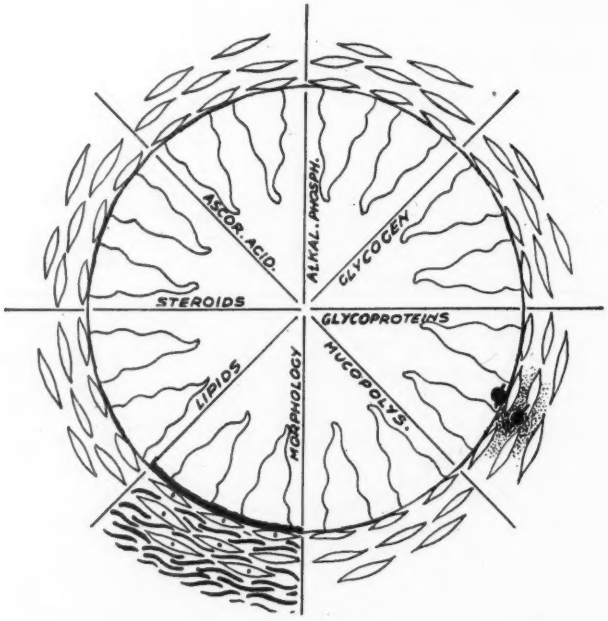


FIG. 3. Klinefelter's syndrome. Tubular lesion, degree II. (For key to nomenclature *see* Fig. 1.) Marked increase in hyalinized collagenous fibers and fibroblasts in the tunica propria; increased thickening of basal membrane. Negative reactions to mucopolysaccharide tests in tunica propria, but reactions to glycoprotein tests are more intense in basal membrane. The Sertoli cells have lost their cytochemical reactions.

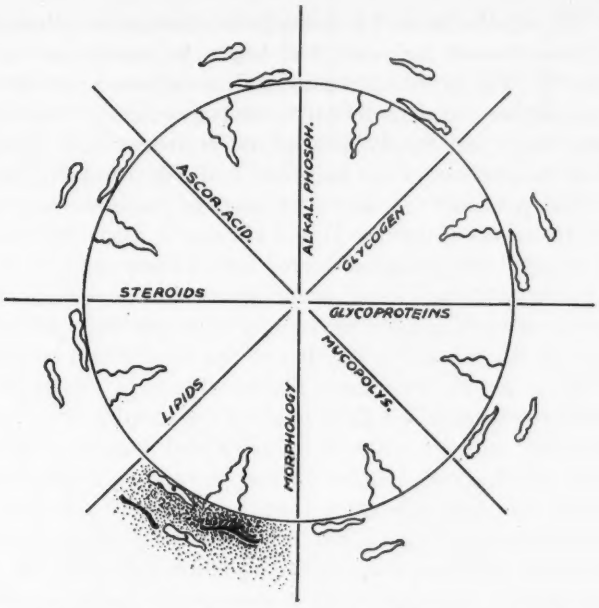


FIG. 4. Klinefelter's syndrome. Tubular lesion, degree III. (For key to nomenclature *see* Fig. 1.) Replacement of collagen fibers by a hyalinized collagenous substance in the tunica propria. Partial disappearance of basal membrane. In other tubular walls the hyalinized material shows a process of disintegration; negative reaction to glycoprotein test in basal membrane and tunica propria. Atrophy of fibroblasts and Sertoli cells.

1. *Tubules:* A sequential progression of changes was observed in the tubules. This sequence has been divided into three stages, as follows: lesion degree I, lesion degree II, and lesion degree III (Figs. 2, 3, and 4).

a) *Tubule wall.* In the basal membrane and tunica propria the changes in the morphologic and histochemical picture of the lesions which we call degrees I, II, and III, could be found at the same time in one patient. Lesion degree I (Fig. 2) was evidenced by tubules with some germinal epithelium which was involuted. Even in tubule walls which had a normal morphologic appearance, disturbances of the histochemical features were observed; less intense reactions were shown to mucoprotein tests, particularly in the tunica propria. The basal membrane and tunica propria showed increased thickening, and a greater number of reticular fibers and some collagen fibers were also recognizable. Lesion degree II (Fig. 3) consisted of a collagenous transformation with marked hyalinization of the tubular wall. The reactions to mucoprotein tests were negative in the tunica propria, whereas they increased slightly in the basal membrane; reticular

fibers were less in number and a collagenous amorphous substance, palely stained by mucoprotein methods, had begun to appear among them. In lesion degree III (Fig. 4) the hyalinized wall undergoes a process of dissolution; the persistent basal membrane swells, weakens in its affinity for mucoprotein tests, and finally disappears. Reticular and collagen fibers of the tunica propria could not be found and only the amorphous collagenous material persisted; it, however, showed variable degrees of disintegration. In lesions of degrees II and III elastic fibers disappeared from the tunica propria; only in lesion degree I were there some elastic fibers in the tunica propria but they were less numerous than in normal adult testes. The hyaluronidase test, applied previously to mucoprotein techniques, had no effect on the histochemical staining of the basal membrane and tunica propria in all the lesions described. The fibroblasts of the tubule wall lost their normal cytochemical qualities in all of the tubules; reactions to alkaline phosphatase, ascorbic acid and lipids yielded negative results, even in those tubules which showed rather normal morphologic and histochemical features of the remaining elements (beginning on lesion degree I). In the tubules with morphologically normal walls the morphologic characteristics of the fibroblasts were not altered, but in lesion degree II the fibroblasts were hypertrophied and were more numerous. In lesion degree III they were atrophic.

b) *Sertoli cells* (Figs. 2, 3, and 4). The number of these cells varied greatly; a greater number were found in tubules with morphologically normal walls, where they showed a trophic appearance. In tubules with lesions of degrees II and III they showed a tendency to disappear, and as a rule they showed atrophic features or signs of involution. Histochemical reactions were generally negative for lipids, glycogen and ascorbic acid, but in some cells of normal appearance, or in progressive atrophy, such reactions were slightly positive.

c) *Germinal epithelium* (Figs. 2, 3, and 4). This was generally absent or greatly damaged. Only a few tubules were found in which germinal epithelium had developed to the primary spermatocyte stage and occasionally to spermatids; these tubules showed a wall with all the characteristics of the beginning of what has been called here, lesion degree I, and usually they were located near Leydig cells with more or less normal secretory activity. Cytochemical reactions to alkaline phosphatase were slightly positive in the spermatogonia and primary spermatocytes; reactions to glycogen were positive only in the spermatogonia. Occasionally it was possible to see infantile-like tubules, with one or two rows of epithelial cells not yet differentiated, and a thin basal membrane and tunica propria.

2. *Intertubular connective tissue:* The Leydig cells showed three different morphologic and cytochemical stages: a) Large nodule formation of adenom-

atous appearance, composed of hypertrophic Leydig cells (Fig. 5) with marked signs of immaturity and proliferation, and few of secretory activity. These cells were usually localized near tubules with lesions of degrees II and III. Most of them showed a homogeneous cytoplasm and were slightly granular in the perinuclear zone; as a rule the nuclei were large with rather visible nucleoli; at times these cells were binuclear. Most of these nodular cells reacted positively for alkaline phosphatase and they

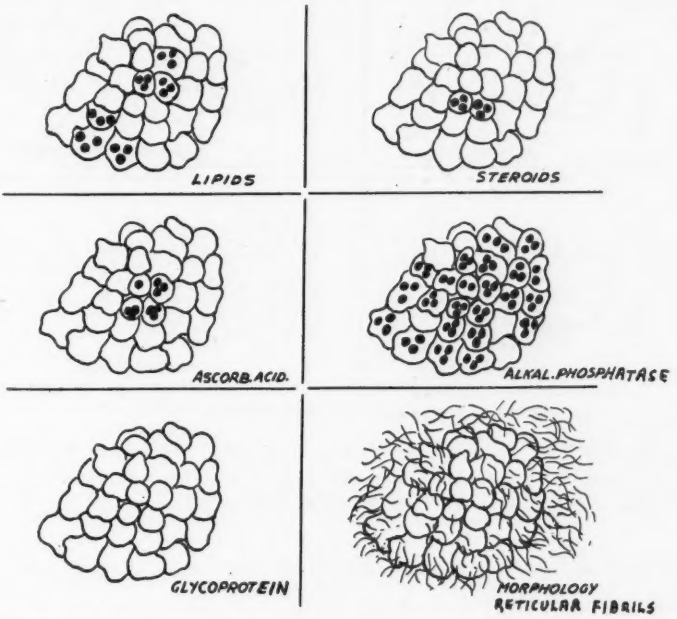


FIG. 5. Klinefelter's syndrome. Large nodular formation of Leydig cells with adenomatous appearance, composed of hypertrophic cells with marked signs of proliferation. Only a few of them show cytochemical signs of secretory activity.

had an intense basophilic reaction. They were concentrically surrounded by an intense network of reticular fibers. They did not show ascorbic acid, cholesterol or its esters, or mucoprotein granules, as do normal Leydig cells. Sometimes tubules with a hyalinized wall were encountered inside the nodules. b) Leydig cells distributed diffusely near tubules with morphologically normal walls (Fig. 6), or with peritubular sclerosis. Most of them showed signs of proliferation but none of secretory activity. The network of reticular fibers was less dense than in the nodular stage. c) Leydig cells with scarce signs of secretory activity and in various stages of transition to fibroblasts were observed near tubules with lesions of degrees

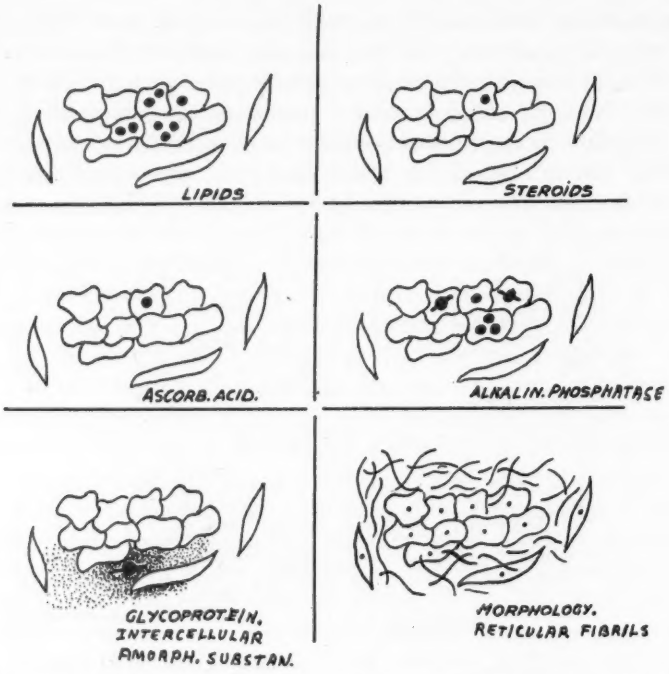


FIG. 6. Klinefelter's syndrome. Leydig cells distributed diffusely in the intertubular connective tissue. The greater portion of them are in a state of proliferation and show cytologic signs of inmaturity. Very few of them show secretory activity.

II and III (Fig. 7), and particularly near tubules with their walls in the process of disintegration.

Mingled with the nodular as well as diffuse stages of Leydig cells, were a few groups of Leydig cells with a trophic appearance which sometimes presented cytochemical features of secretory activity (Figs. 5 and 6), positive reactions for lipids, cholesterol esters and ascorbic acid and negative reactions for both alkaline phosphatase and basophilic staining. These were usually found between or near tubules with morphologically normal walls. The rest of the intertubular connective tissue was filled with reticular and collagen fibers, and fibroblasts in more or less intense proliferation. These fibroblasts yielded negative histochemical reactions and had cytoplasmic basophilia. The intercellular substance was diminished compared with normal adult testes.

Individually the cases presented certain differences. For example, in Patients 1 and 5, tubular lesions of degrees I and II predominated and the connective intertubular tissue showed adenomatous nodules of Leydig

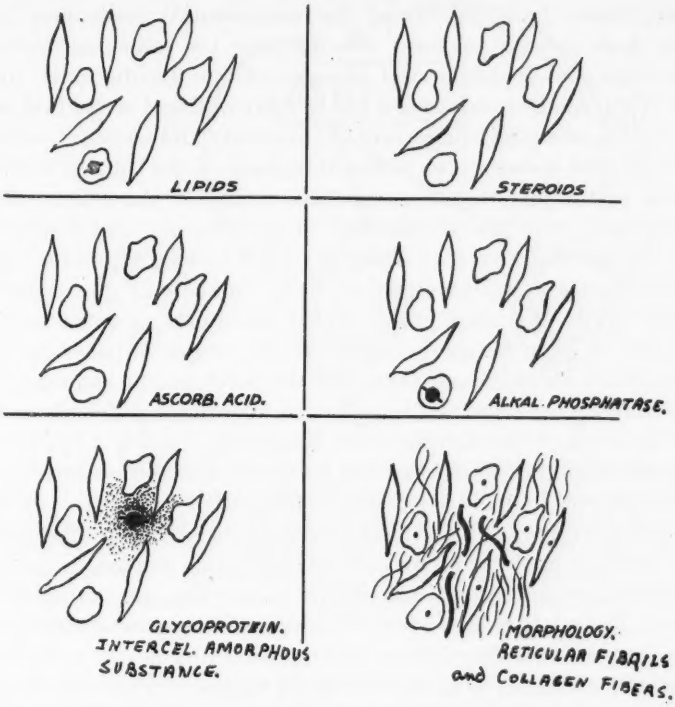


FIG. 7. Klinefelter's syndrome. Leydig cells in transition to fibroblasts. None of them show the cytochemical reactions of secretory activity.

cells. In Patients 2 and 4, tubular lesions of degrees II and III predominated, and in the intertubular tissue the Leydig cells were diffusely distributed. Patient 3 showed a predominance of lesion degree III in the tubules and proliferation of fibroblasts in the intertubular connective tissue.

DISCUSSION

The Klinefelter syndrome is a primarily testicular hypogonadism which apparently starts at puberty and involves all the elements of the testes. The patients are sterile, and the clinical manifestations of androgenic deficiency are not always very noticeable.

In this study we wanted to determine: 1) the pathogenic process responsible for the testicular lesions, and 2) whether all the histologic structures of the testes fail simultaneously, or whether there is some sequence in the pathologic development. With this in mind, an investigation was carried out on a histochemical basis, and the interpretations given here are based

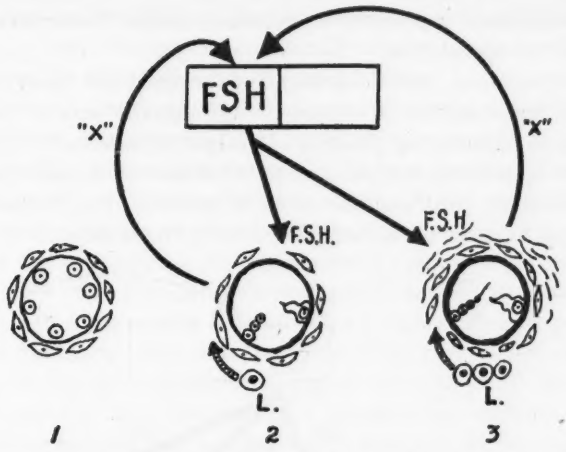
principally upon the specificity of the histochemical techniques applied.

In the cases studied we want to emphasize the following findings: 1) the histologic and histochemical changes of the Leydig cells, and the changes in the connective tissue of the tubular wall and of the intertubular spaces, which are equally important; 2) precocity and constant uniformity of the functional disturbances in the fibroblasts of the tubular wall and in fibroblasts of the intertubular connective tissue; 3) the loss by the connective structures of the tubule wall of morphologic and histochemical features; 4) the disappearance of the germinal epithelium and the persistence of the Sertoli cells, correlated with the intensity of the alterations in the tubular walls. It is quite probable that the alteration in the tubule wall is accompanied by a change in its permeability which impedes the passage of the necessary nutritive substances for the development and maintenance of the germinal epithelium and Sertoli cells; 5) noticeable disturbance of the specific function of the Leydig cells. Excepting the few Leydig-cell foci with morphologic and histochemical features of secretory activity, most of the intertubular connective tissue is filled with immature Leydig cells with signs of proliferation and many forms in transition to fibroblasts.

Some findings in the connective tissue of certain organs, under various physiopathologic conditions, are related to our histogenic theory of this syndrome. The relationship between the intercellular ground substance and the development of collagen fibers and basal membranes, is a phenomenon frequently encountered in many connective tissues in which morphogenesis is related to the activity of fibroblasts (Gersh and Catchpole (28)), (Mancini and Bacarini (29)). Besides, this tissue seems to respond to certain hormones in the maintenance of their morphologic and histochemical characteristics (Tabenhaus and Amromin (30)), (Ludwig and Boas (31)), (Duran-Reynals *et al.* (32)), (Mancini and Lustig (33)), (Mancini, Garberi and de la Balze (34)).

A previous study of the testes of newborn, prepuberal and adult normal subjects (Mancini, Nolasco and de la Balze (35)) has led us to suppose that fibroblasts are related to the production of amorphous substance and connective fibers in the testes; it seems likely that in this process the fibroblasts are the end organs of an hormonal action. This concept has been further substantiated by our observation of the fact that development and organization of the connective tissue of the tubular wall and of intertubular spaces reaches maturity only at puberty, simultaneously with the appearance of adult Leydig cells. All this induces us to express the opinion that the structure and function of the tubular wall depends on the functional normality of the Leydig cells.

On the basis of our findings we propose the following hypothesis for the pathogenic interpretation of the Klinefelter syndrome (Figs. 8 and 9). At



FIGS. 8 and 9. In both figures the top square shows the anterior pituitary gland with reference only to FSH, and not to other functions. In the intertubular connective tissue, Leydig cells and fibroblasts are the only elements represented. FSH = follicle-stimulating hormone; "X" = hypothetical hormone which originates in the tubule and participates in the regulation of FSH; L = mature Leydig cells; I.L. = immature Leydig cells; (⟸) = normal androgenic local action; (⇠) = decreased or absent androgenic local action; (←) = normal FSH or normal "X" hormone; (⇐) = increased FSH; (←--) = absent "X" hormone.

FIG. 8. A normal seminiferous tubule is represented, developing during puberty and passing from the infantile status to adult condition. In Klinefelter's syndrome this happens only in very few tubules. 1: corresponds to an infantile tubule surrounded by fibroblasts and containing undifferentiated cells. 2: corresponds to a tubule in the beginning of puberty. 3: corresponds to an adult tubule which has otherwise attained all the characteristics of adulthood. This evolution of the tubules appears to be in great part the result of the combined stimulation of FHS and the androgenic secretion of the neighboring mature Leydig cells.

puberty a disturbance in the normal development and differentiation of the Leydig cells may occur, presumably due to the lack of, or some qualitative change in LH or to some disturbance in the capacity of the Leydig cells to respond adequately to normal LH; few of them are transformed into adult secretory cells; the rest remain immature, but will proliferate. The result is a decrease in a local and general androgenic support needed to maintain the normal function of the fibroblasts of the tubular wall and of the intertubular connective tissue. This causes a disorderly proliferation of these cells, with abundant production of reticular fibrils and collagen fibers, particularly in the wall of the tubules and also in the intertubular connective tissue. The tubular wall suffers changes which impede the normal nutrition needed for the development and maintenance of the germinal epithelium and Sertoli cells. Thus the condition of the tubular wall, espe-

cially its fibroblasts, represents a secondary sexual characteristic, such as the prostate or epididymis.

On the other hand, there is evidence to support the theory that FSH is an essential factor in the development and maintenance of the seminiferous tubules at the time of puberty. It might be assumed that in cases of Klinefelter's syndrome, the seminiferous tubule receives adequate gonadotropic stimulation (FSH) at the onset of puberty, but because of the absence of local androgenic stimulation due to the failure in the normal de-

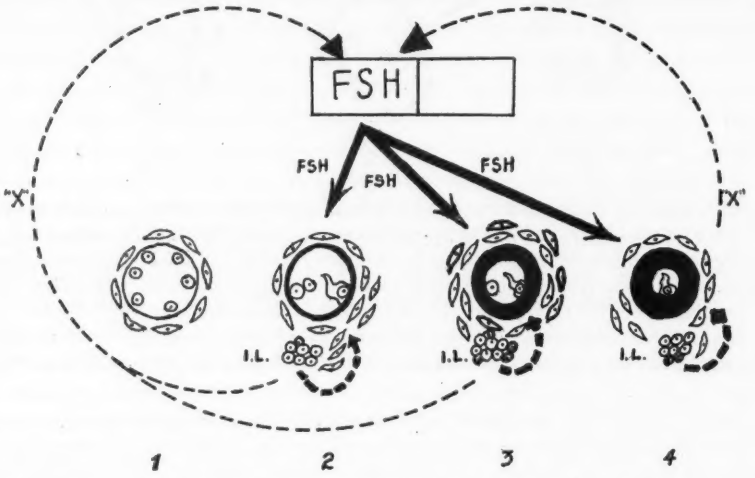


FIG. 9. Represents what may happen in most of the tubules in Klinefelter's syndrome. 1: corresponds to infantile tubules. 2, 3, and 4: correspond to tubules which are growing and developing during puberty and under the action of FSH but without the local androgenic stimulation, because the neighboring Leydig cells are immature. The transition from the infantile to the adult status has not occurred. This shows the sequence of the lesions as they would be found in most tubules in Klinefelter's syndrome. This sequence is what is designated in this paper as lesions of degrees I, II, and III (*see* Figs. 2, 3 and 4).

velopment of Leydig cells, the tubular wall and the germinal epithelium experience the pathologic changes already described; only tubules close to neighboring adult Leydig cells reach maturity. Consequently, we think that the increase in gonadotropin excretion is a consequence of, not the cause of, testicular alterations, in the Klinefelter syndrome.

This sequence of effects may explain the fact that in one testis there may be found simultaneously a few tubules with normal adult features, tubules with entirely infantile characteristics, and tubules with the greater portion showing lesions of degrees I, II, and III (Figs. 2, 3, and 4). The presence of infantile tubules may be explained as a result of the lack of development

of lesions or germinal epithelium. In other words, on the basis of our theory, the evolution of the disease will eventually result in the development of lesions in the walls of the infantile-like tubules.

The existence of tubules which have retained features closely resembling normal conditions, is interpreted as a result of the synergic action of FSH and the local action of testosterone produced by a few foci of neighboring mature Leydig cells, which show cytochemical signs of secretory activity.

Our hypothesis that the endogenous testosterone maintains the seminiferous tubules just as it maintains other elements of the male reproductive system, coincides with the alternate explanation given by Howard *et al.* (4), and is in accordance with that of other authors. Regarding this hypothesis, the following clinical observations deserve attention. In our patients, foci of Leydig cells with normal morphologic and histochemical features were usually found near the few tubules that were less damaged. Howard *et al.* (4) proved in a patient with idiopathic eunuchoidism (with low FSH) that the tunica propria, previously thickened, had become thin some time after administration of 1,050 mg. of testosterone. Bartter *et al.* (36) administered chorionic gonadotropin to subjects affected by hypogonadotropic eunuchoidism and were able to observe that normal characteristics developed in the Leydig cells, and the tubular wall also showed favorable changes. Nevertheless, Heller *et al.* (37) observed that daily doses of 25 mg. of testosterone propionate in normal men caused a marked hyalinization of the basal membrane and tunica propria of the tubules.

Referring to experimental observations in animals, we do not intend to review all the previous work on the subject, but we consider it worth noting that testosterone administered to rats has an inhibitory as well as a stimulating action, related to the dose, upon the weight and spermatogenesis of the testes (Ludwig (38)). In animals, the stimulating action seems to correspond to a direct effect upon the testes, either by injected hormone or local application of testosterone pellets.

If it is accepted that the seminiferous tubule is a secondary sexual characteristic, the discrepancy existing in the Klinefelter syndrome between the marked tubular changes and the less intense manifestations of androgenic deficiency in the remaining sexual characteristics, is striking. An acceptable explanation for this apparent paradox might be to assume that to secure their normal development and maintenance, the tubules would need the local action of testosterone (mature Leydig cells close to the tubules) in addition to its general action.

The testes of this syndrome present specific histologic characteristics in the intertubular connective tissue, where there is no inflammatory or vascular lesion. It may be possible to find similar tubular wall lesions in other conditions, for instance, in cases of postpuberal hypogonadism and

of mumps orchitis. In the chronic cases of mumps orchitis which we have had under observation, most of the Leydig cells present normal morphologic and histochemical features. Besides, in the Klinefelter syndrome the peritubular sclerosis is different from that found in old age, because there is no increase in the amount of elastin.

We should mention here that Reifenstein (39) cited a case which would support the genetic etiology of the Klinefelter syndrome, *viz.*, a family in which various members were affected. Moreover, cases of myotonic dystrophia have been reported, in which testicular histology revealed pictures similar to those presented by the Klinefelter syndrome (Nadler *et al.* (40)).

SUMMARY

Five cases of Klinefelter's syndrome have been studied, in which were found the clinical and hormonal characteristics described by other authors. Morphologic and histochemical studies of the testicular biopsy material have been made in order to obtain a histophysiologic basis for a pathogenic interpretation. From the histologic point of view, the lesions of the intertubular connective tissue were as marked as those of the tubular wall and germinal epithelium. Clinically, however, sterility was always present, even when manifestations of androgenic deficiency were not apparent. As a result of the clinical, hormonal and histologic studies on our cases, the following pathogenic interpretation is proposed. At puberty a disturbance in the normal development and differentiation of the Leydig cells occurs, so that few of them reach the adult secretory stage. This deficiency in androgenic action (particularly local action) causes abnormal fibroblast function in the tubular wall and in the intertubular connective tissue. Fibroblasts proliferate, produce abundant reticulin fibrils and later, collagen fibers; finally hyalinization of the tubular wall occurs. The progressive lesion of the wall leads to atrophy of the germinal epithelium and Sertoli cells. Secondarily there is an increase in hypophyseal gonadotropins.

Acknowledgments

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REFERENCES

1. Klinefelter, H. F., Jr.; Reifenstein, E. C., Jr., and Albright, F.: Syndrome characterized by gynecomastia, aspermatogenesis without a-Leydigism and increased excretion of follicle-stimulating hormone, *J. Clin. Endocrinol.* **2**: 615, 1942.
2. Heller, C. G., and Nelson, W. O.: Hyalinization of the seminiferous tubules associated with normal or failing Leydig-cell function. Discussion of relationship to eunuchoidism, gynecomastia, elevated gonadotrophins, depressed 17-ketosteroids and estrogens, *J. Clin. Endocrinol.* **5**: 1, 1945.

3. Nelson, W. O., and Heller, C. G.: Hyalinization of the seminiferous tubules associated with normal or failing Leydig-cell function. Microscopic picture in the testis and associated changes in the breast, *J. Clin. Endocrinol.* **5:** 13, 1945.
4. Howard, R. P.; Sniffen, R. C.; Simmons, F. A., and Albright, F.: Testicular deficiency: a clinical and pathologic study, *J. Clin. Endocrinol.* **10:** 121, 1950.
5. Gorbman, A.: Ultrafiltration of urine for collection and biological assay of excreted hypophyseal hormones, *Endocrinology* **37:** 177, 1945.
6. Klinefelter, H. F., Jr.; Albright, F., and Griswold, G. C.: Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis, *J. Clin. Endocrinol.* **3:** 529, 1943.
7. de la Balze, F. A.; Arrillaga, F. C., and Carcano, C. A.: Determinación de gonadotrofinas hipofisarias extraídas de la orina por el ultrafiltro. Resultados obtenidos en un grupo de 33 testigos normales, *Medicina* **11:** 71, 1951.
8. Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric determination of substances containing grouping —CH_2CO— in urine extracts as indication of androgen content, *Biochem. J.* **32:** 1312, 1938.
9. Charny, C. W., and Meranze, D. R.: Testicular biopsy; further studies in male infertility, *Surg., Gynec. & Obst.* **74:** 836, 1942.
10. Mancini, R. E.: Reacción del yodo para la investigación histoquímica del glucógeno, *Arch. Soc. argent. anat.* **26:** 420, 1944.
11. Hotchkiss, R. D.: A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations, *Arch. Biochem.* **6:** 131, 1948.
12. McManus, J. F. A.: Histological demonstration of mucin after periodic acid, *Nature* **158:** 202, 1946.
13. Etcheverry, M. A., and Mancini, R. E.: Reacción fotoquímica para la investigación de polisacáridos en los tejidos, *Rev. Soc. argent. de biol.* **24:** 155, 1948.
14. Lison, L.: Histochimie animal. Paris, Gauthiers-Villars, 1936.
15. Hale, C. W.: Histochemical method for hyaluronic acid, *Nature* **157:** 802, 1946.
16. Cain, A. J.: The use of Nile blue in the examination of lipoids, *Quart. J. Microsc. Sci.* **88:** 383, 1947.
17. Bennett, H. S.: The life history and secretion of the cells of the adrenal cortex of the cat, *Am. J. Anat.* **67:** 151, 1940.
18. Kabath, E. A., and Fursth, J.: Histochemical study of the alkaline phosphatase in normal and neoplastic tissues, *Am. J. Path.* **17:** 305, 1941.
19. Deane, H. W., and Morse, A.: The cytological distribution of ascorbic acid in the adrenal cortex of the rat under normal and experimental conditions, *Anat. Rec.* **100:** 127, 1948.
20. Mancini, R. E.; Nolazco, J., and de la Balze, F. A.: Histochemical study of the normal adult human testis, *Anat. Rec.* 1952. (In press.)
21. Mancini, R. E.: Histochemical study of glycogen in tissues, *Anat. Rec.* **101:** 149, 1948.
22. Sosa, J. M.: Vitamin C microscopic demonstration and Golgi apparatus, *Exper. Cell Research* **3:** 184, 1952.
23. Leduc, E. H., and Dempsey, E. W.: Activation and diffusion as factors influencing the reliability of the histochemical method for alkaline phosphatase, *J. Anat.* **85:** 305, 1951.
24. Dempsey, E. W., and Wislocki, G. B.: Histochemical contributions to physiology, *Physiol. Rev.* **26:** 1, 1946.

25. Lillie, R. D.: Further exploration of the HIO_4-Schiff reaction with remarks on its significance, *Anat. Rec.* **108:** 259, 1950.
26. Leblond, C. P.: Distribution of periodic-acid-reactive carbohydrates in the adult rat, *Am. J. Anat.* **86:** 1, 1950.
27. Mancini, R. E.: Investigación histoquímica de los mucopolisacáridos, *Rev. Soc. argent. de biol.* **26:** 139, 1950.
28. Gersh, L., and Catchpole, G. W.: The organization of ground substance and basement membrane and its significance in tissue injury, disease and growth, *Am. J. Anat.* **85:** 457, 1949.
29. Mancini, R. E., and Bacarini, E.: Mucoproteínas en el desarrollo del tejido conectivo adulto y embrionario, *Rev. Soc. argent. de biol.* **27:** 27, 1951.
30. Tabenhaus, M., and Amromin, G. D.: Influence of steroid hormones on granulation tissue, *Endocrinology* **44:** 359, 1949.
31. Ludwig, A. W., and Boas, N. F.: The effect of testosterone on the connective tissue of the comb of the cockerel, *Endocrinology* **46:** 291, 1950.
32. Duran-Reynals, F.; Bunting. H., and Van Wagenen, G.: Studies on the sex skin of Macaca mulatta, *Ann. New York Acad. Sci.* **52:** 1006, 1950.
33. Mancini, R. E., and Lustig, E. S.: Efecto de la cortisona y desoxicortisona sobre el cultivo de fibroblasto "in vitro," *Rev. Soc. argent. de biol.* 1951. (En prensa.)
34. Mancini, R. E.; Garberi, J. C., and de la Balze, F. A.: Mucroproteinas del tejido conectivo de la piel y de la sangre en el mixedema humano, *Rev. Soc. argent. de biol.* 1951. (En prensa.)
35. Mancini, R. E.; Nolazco, J., and de la Balze, F. A.: Desarrollo y organización del tejido conectivo en el testiculo humano, *Rev. Soc. argent. de biol.* **27:** 156, 1951.
36. Bartter, F. C.; Sniffen, R.; Simmons, F., and Albright, F.: Therapy of eunuchoidism with chorionic gonadotropin: aqueous solution compared with an oil and beeswax suspension (abstract) *J. Clin. Endocrinol.* **11:** 768, 1951; also Bartter, Sniffen, Simmons, Albright and R. P. Howard: Effects of chorionic gonadotropin (APL) in male "eunuchoidism with low follicle stimulating hormone": aqueous solution versus oil and beeswax suspension, *J. Clin. Endocrinol. & Metab.* **12:** (Dec.) 1952. (In press.)
37. Heller, C. G.; Nelson, W. O.; Hill, I. C.; Henderson, E.; Maddock, W. O., and Jungck, E. C.: The effect of testosterone administration upon the human testis, (abstract) *J. Clin. Endocrinol.* **10:** 816, 1950.
38. Ludwig, D. J.: The effect of androgens on spermatogenesis, *Endocrinology* **46:** 453, 1950.
39. Reifenstein, E. C., Jr.: Hereditary familial hypogonadism, *Proc. Am. Fed. Clin. Research* **3:** 86, 1947.
40. Nadler, C. S.; Steiger, W. A.; Troncelleti, M., and Durant, T. M.: Dystrophia myotonica, with special reference to endocrine function (Klinefelter's syndrome), *J. Clin. Endocrinol.* **10:** 630, 1950.

PROGESTERONE IN AQUEOUS CRYSTALLINE SUSPENSION VERSUS PROGESTERONE IN OIL: COMPARISON BY WITHDRAWAL BLEEDING TESTS IN THE HUMAN FEMALE

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IN accord with the recent trend in endocrine replacement therapy toward a depot effect, studies are being reported on comparisons of the relative effects of the various steroid hormones in aqueous crystalline suspension and in oily solution. The literature on this subject is still small and is chiefly limited to work on animals. The following experiments were designed to test the comparative activity of an aqueous crystalline suspension of progesterone, and a solution of progesterone in sesame oil, in elderly postmenopausal women.

Estrogen and progesterone withdrawal bleeding in many patients well past the menopause has been previously demonstrated (1). The criterion established for comparing the biologic activity of various preparations having luteal activity, namely, the capacity to produce withdrawal bleeding under certain dosage schedules, was followed in the present study. The production of uterine bleeding by this method is dependent upon progesterone deprivation in the presence of sustained estrogen influence.

MATERIALS AND METHODS

The postmenopausal women in this investigation had been under a maintained estrogen influence (2 milligrams of estradiol benzoate intramuscularly each week) for more than four years as part of a basic study of hormone replacement in the aged (1, 2). These same women, in addition to the sustained estrogen stimulation, had been the subjects of reports on a) experimental attempts to simulate, under cyclic administration of progesterone, the sequence of events seen in the physiologic menstrual cycle (1), and b) experimental production of irregular endometrial shedding, using varying dosages of progesterone under different schedules (2). The age range was 70–84 years and the average age was 75 years. Women in this age group were chosen for these experiments because of their ready availability and their potential usefulness in the bio-assay of sex hormones

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(3, 4, 5). It is of particular importance that, in previous work with these women, their ability to respond to intramuscular injections of progesterone in oil had already been determined.

The subjects of this study were all aware of the nature of this procedure and were used with their full consent.

During the course of the eleven experiments in this study, 3 of the 6 original subjects were lost to the group because of death or intercurrent surgery. The 2 deaths were not the direct result of chronic or acute disease but were due to the cumulative infirmities of old age.

The crystalline preparation used for comparison consisted of a macrocrystalline suspension containing progesterone crystals of 100 to 300 microns in diameter.[1] The concentration was 25 milligrams per cubic centimeter. The oily solution of progesterone employed as a control medication was the same as in the previously reported assays, the concentration being 10 milligrams per cubic centimeter.[2]

A series was completed of eleven experiments in succession with injectible progesterone (Table 1). Of these, the third, sixth and eleventh involved progesterone in oil and were included merely to demonstrate again, for closer temporal comparison, the responsiveness of these subjects to this form of the hormone. Aqueous crystalline progesterone was employed in all the other tests in varying dosages. The medications were given intramuscularly through a 20-gauge needle each morning during the trial period. During experiments in which a 20-milligram dose was administered daily, injections were given at twelve-hour intervals. It was found impossible to inject the complete dose of the aqueous form through a needle of smaller than 20 gauge because of the size of the suspended crystals.

The objective criterion for determination of effective dosage in this investigation was the phenomenon of withdrawal bleeding. A trial period of medication was begun in each case two to three weeks after the previous test, if no bleeding had ensued. If withdrawal bleeding had been noted following the previous medication in any subject, four weeks elapsed between the test periods to allow re-establishment of a properly estrogen-primed endometrium. The investigation was additionally stabilized by proper spacing of the progesterone-in-oil experiments to cause satisfactory shedding of the endometrium twice, thus obviating possible cumulative hormonal effects of the crystalline suspensions on the endometrium. An endometrial biopsy specimen was obtained before the onset of flow in every case in which the aqueous form was employed and withdrawal bleeding observed. Although multiple biopsies had been performed during pre-

Generously supplied by:

[1] Ciba Pharmaceutical Products, Inc.

[2] Schering Corporation.

vious investigations which involved the use of progesterone in oil, biopsies were repeated following oil administration in the present work. For purposes of constancy, the biopsy findings presented in this paper are all from the same subject.

RESULTS

The clinical results of the eleven experiments comprising the present investigation are listed in Table 1. All patients had withdrawal bleeding in experiments three, six and eleven, in each of which the oil solution had been used. Previous work on this same group of patients (1) had demonstrated

TABLE 1. DOSAGE OF AQUEOUS CRYSTALLINE PROGESTERONE NECESSARY TO PRODUCE WITHDRAWAL BLEEDING

Experiment number	Number of subjects*	Injection medium	Daily dosage† (mg.)	Duration of medication (days)	Total dosage (mg.)	Subjects showing withdrawal bleeding	Endometrial biopsy specimen obtained
1	6	aqueous	10	1	10	0	
2	6	aqueous	10	2	20	0	
3	6	oily	10	3	30	6	×
4	5	aqueous	10	3	30	0	
5	4	aqueous	10	3	30	0	
6	4	oily	20	2	40	4	
7	4	aqueous	20	3	60	4	×
8	4	aqueous	10	5	50	3	×
9	4	aqueous	20	2	40	3	×
10	3	aqueous	10	4	40	2	×
11	3	oily	10	3	30	3	

* All subjects were receiving α-estradiol benzoate, 2 mg. per week, as basic medication.

† All medication was given intramuscularly.

withdrawal bleeding of all subjects following 5 milligrams of progesterone in oil daily for three days, and in more than half the subjects after as little as a single injection of 5 milligrams in oil. At that time they were receiving the same basic estrogenic medication as in the present study.

Using the aqueous suspension, no bleeding was noted until experiment seven, in which all patients experienced withdrawal bleeding after receiving 60 milligrams in three days. This was the lowest total dosage of aqueous progesterone which was associated with bleeding in all patients under therapy. When aqueous progesterone was administered in a daily dose of 10 milligrams for five days (a total dosage of 50 milligrams) 3 of 4 patients demonstrated withdrawal bleeding. Three of 4 patients flowed with 40

milligrams of the aqueous suspension (20 milligrams daily for two days), and 2 of 3 subjects flowed with 10 milligrams given daily for four days. At the next lowest dosage, 30 milligrams in three days, as in experiment four and repeated in experiment five, none of the patients experienced withdrawal bleeding.

Referring again to the previous work on this same group of subjects (1), Table 2 of that paper lists a series of experiments employing a solution of progesterone in sesame oil. These tests were similar to those of this paper

TABLE 2. PROGESTERONE WITHDRAWAL BLEEDING: COMPARISON OF AQUEOUS AND OILY MEDIA

Injection medium	Daily dosage* (mg.)	Duration of medication** (days)	Total dosage (mg.)	Number of subjects tested	Withdrawal bleeding	
					Number of subjects	Percentage
Oily	10	1	10	9	9	100
Aqueous	10	1	10	6	0	0
Oily	10	2	20	9	9	100
Aqueous	10	2	20	6	0	0
Oily	10	3	30	18	18	100
Aqueous	10	3	30	9	0	0
Oily	10	4	40	9	9	100
Aqueous	10	4	40	3	2	67
Oily	10	5	50	18	18	100
Aqueous	10	5	50	4	3	75

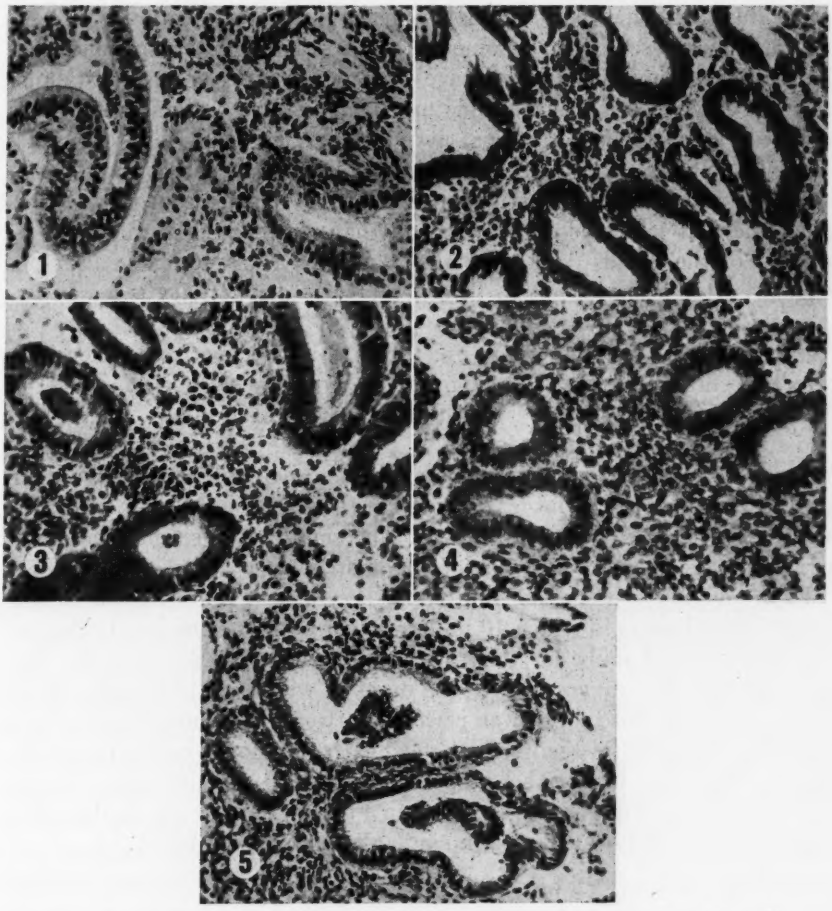
* All medication was given intramuscularly.

** All subjects were receiving α-estradiol benzoate, 2 mg. per week, as basic medication.

and afford additional data for the comparison of the aqueous with the oily form of progesterone. This is more clearly demonstrated in Table 2, which lists in order of increasing total dosages the comparable tests of Table 2 of the previous work and Table 1 of this paper. Only those experiments are compared in which the daily dosage was 10 milligrams. As shown in the present Table 2, all patients receiving at least 10 milligrams of progesterone in oil, regardless of the total dosage, demonstrated withdrawal bleeding. The 6 subjects of experiment three and the 3 subjects of experiment eleven of this paper (Table 1) are added to the 9 subjects of the previous work who received 10 milligrams in oil daily for three days, making a total of 18. The comparative tests of the aqueous form with the same total dosage of

30 milligrams are seen in experiments four and five of this paper, yielding a total of 9 individual tests, in none of which was withdrawal bleeding experienced.

Figures 1 through 4 are microphotographs of endometrial biopsy specimens taken just prior to the onset of bleeding in experiments seven through ten, respectively, of the present study (Table 1). Figure 1, associated with



Microphotographs of endometrial biopsy specimens following administration of:
FIG. 1. Aqueous progesterone, 60 mg. (20 mg. ×3 days) 280x.
FIG. 2. Aqueous progesterone, 50 mg. (10 mg. ×5 days) 280x.
FIG. 3. Aqueous progesterone, 40 mg. (20 mg. ×2 days) 280x.
FIG. 4. Aqueous progesterone, 40 mg. (10 mg. ×4 days) 280x.
FIG. 5. Oily progesterone, 30 mg. (10 mg. ×3 days) 280x.

the 60-milligram total dosage of crystalline progesterone, shows a fairly well stimulated secretory endometrium. There is generally a middle to basal position of the epithelial cell nuclei, with significant evidence of vacuolization on either side of the nucleus. The stroma is similarly stimulated with moderate edema and evidence of early blood extravasation. Comparing the glands to those of a physiologically menstruating endometrium, they present a moderate, or relatively immature, secretory phase rather than the usual fully-stimulated, mature, secretory picture.

Figure 2 represents the endometrium just prior to bleeding, following the administration of a total dosage of 50 milligrams of the crystalline progesterone preparation. This picture demonstrates a significantly less advanced stage of progestational activity than noted in Figure 1. The epithelial cell nuclei generally are middle or luminal in position and basal vacuoles are seen in only occasional cells. There is very little stromal edema and somewhat less blood extravasation than shown in Figure 1. Cross-sections of the glands demonstrate invagination to some extent, but no lengthwise tortuosity is noted. In general, secretory activity is markedly restricted.

Figure 3 shows the endometrial picture in a biopsy specimen taken just before bleeding, following 40 milligrams of crystalline progesterone over a two-day period. The picture is much the same as that in Figure 2. Figure 4, also associated with a total dose of 40 milligrams, but administered over four days, shows only good estrogen effects and no evidence of any luteal effects. The thick hyperchromatic nuclei of the epithelial cells are centrally placed. No stromal edema is present. The small ovoid simple glandular arrangment is one of a nonsecretory endometrium.

Figure 5 shows an endometrial biopsy specimen taken after one of the control experiments in which 10 milligrams of progesterone-in-oil solution was administered daily for three days. As with the other biopsies, this specimen also was taken prior to the onset of withdrawal bleeding. Relatively speaking, this biopsy was performed twenty-four hours earlier than the biopsy depicted in Figure 1, so blood extravasation is not as marked a feature. However, in all other respects, including epithelial cells, stroma, and glands, the biopsy findings shown in Figures 1 and 5 are similar. The overall picture of both suggests a similar progestational effect.

HISTORY AND DISCUSSION

The physicochemical properties of any drug or hormone preparation largely determine its rate of absorption and duration of effect. Considerable variation in dosage has been observed in attempts to produce similar effects using one hormone in different substrates. In general, investigations to date have suggested that a crystalline steroid hormone in aqueous

suspension in a lesser dosage results in more profound effects than does the same hormone in an oil medium. This is attributed to the depot effect of the crystals, *i.e.*, better utilization due to slower absorption over longer periods of time.

Meier *et al.* (6), employing the life maintenance test following adrenalectomy in multiple animal species, compared the aqueous to the oil forms of desoxycorticosterone acetate. They ascertained the duration and intensity of effect following a single subcutaneous administration of the same dose of each form. Concluding that the crystalline suspension definitely exerted a more prolonged effect and better utilization than did the oil solution, they additionally stressed the variation in effect that occurs with change in crystal size, noting that size would be limited by needle diameters. Free estrogens in aqueous suspensions were first demonstrated by Richards, Spruth, and Russell (7) to induce in mice and rats an estrus that lasted many times longer than with the oil solution. Meischer *et al.* (8), in their excellent study of this subject, worked with castrated rats and developed a new test for progesterone based on the repression of continuous estrus induced by estrogen administration. By this test they were able to demonstrate very clearly in these animals the more marked and more prolonged effect of crystalline suspensions as compared to oily solutions of progesterone. Crystals of varying sizes were used and were classified as fine, medium, or coarse, the latter size corresponding roughly to the type of crystal used in the work reported in this paper. The coarse crystals proved to be more effective than the medium or fine crystals. They stressed further that crystalline suspensions, although not quite as long-acting as pellets, may be sterilized much more easily without altering the effective hormone, and may also obviate the necessity of a surgical procedure.

Koref and Engle (9) worked with microcrystals of progesterone which apparently passed fairly easily through 20-gauge and 21-gauge needles. Using a single subcutaneous injection, they compared the activity of aqueous suspensions to that of oil solutions by direct uterine biopsies in infantile female rabbits primed with estrone. From their results with these animals they concluded that, for obtaining a minimum effect by a single injection, progesterone in microcrystals is at least four times as active as in oil solution. Also working with rabbits, Chang (10) compared aqueous, oil, and pellet progesterone preparations, using as his criterion the maintenance of pregnancy in rabbits in the absence of corpora lutea. By his methods, the best maintenance occurred with single injections of 25 milligrams of a macrocrystalline suspension two or three times, at eight- to ten-day intervals. Although the dosage of progesterone in oily solution was usually somewhat smaller than that in aqueous suspension, it is of note that daily injections of the oily form were continued over three to four

weeks, whereas in a similar period of time only two or three administrations of the crystalline suspension were necessary.

In studies on the human female, Freed and Greenhill (11) and Joel (12) treated the menopausal syndrome with crystalline estrogenic suspensions. This therapy, according to their conclusions, showed apparent marked superiority over treatment with either the oily solution or the oily suspension of the same hormone. Long and Bradbury (13) were able to induce and maintain decidual changes, using both crystalline estrogen and progesterone, but did not make comparative studies with the oil forms.

The experiments reported in this paper are in contrast to other studies on the same subject published in the literature to date. As already noted, the bulk of the previous work has been performed in animals and not in the human. Experimental work in animals, especially in the field of endocrinology, at best can only be suggestive of possible similar responses in the human. Speculations derived from animal experimentation are important chiefly in the stimulus they provide for further work in the human before definite conclusions can be formed.

In the human, the comparative tests of aqueous suspensions versus oily solutions of progesterone for relief of the menopausal syndrome (11, 12) have depended entirely upon subjective signs. The conclusions arising from the studies by Long and Bradbury (13), as just described, were the result of objective findings in the human subject. However, no comparison was made with the same endocrine products in oily solution.

The present study is an attempt to compare in the human subject the effects of an aqueous crystalline suspension of progesterone to the effects of a solution of progesterone in oil, by means of objective findings (withdrawal bleeding and endometrial biopsy). The principal criterion employed as a standard for comparison, *i.e.*, the phenomenon of withdrawal bleeding, apparently has not been used heretofore.

CONCLUSIONS

The results of these experiments are at variance with the conclusions of other work comparing the effects of oily and of aqueous crystalline hormone preparations. From these results it would seem that, employing the phenomenon of progesterone withdrawal bleeding in association with an estrogen-primed endometrium, aqueous crystalline suspensions of progesterone are much less effective in the human than are oily solutions of the same hormone. Clinically, the oily solution is at least four times as effective, for only 5 milligrams in oil daily for three days produced withdrawal effects in all subjects as shown by previous work on this same experimental group (1), whereas 20 milligrams of aqueous suspension daily for three days was necessary before all subjects experienced withdrawal bleeding. From a

histologic viewpoint, the oily form is about twice as effective. The endometrial biopsy findings prior to withdrawal bleeding, after a total dosage of 30 milligrams of the oily preparation (10 milligrams a day) over a three-day period, are similar to those noted after 60 milligrams of the crystalline form (20 milligrams a day) over a three-day period.

SUMMARY

Progesterone administered in oily solution has been shown to be significantly more effective in the production of withdrawal bleeding in the human subject than is progesterone in aqueous crystalline suspension using coarse crystals. In addition, a more advanced luteal effect is demonstrable by biopsy in the endometria stimulated by progesterone in oily solution than in the endometria stimulated by progesterone in aqueous crystalline suspension.

REFERENCES

1. MASTERS, W. H., and ALLEN, W. M.: Female sex hormone replacement in the aged woman, *J. Gerontol.* **3:** 183–190 (July) 1948.
2. MASTERS, W. H., and MAGALLON, D. T.: The experimental shedding of the endometrium, *Am. J. Obst. & Gynec.* **59:** 970–978 (May) 1950.
3. MASTERS, W. H., and MAGALLON, D. T.: Hormone replacement therapy in the aged female—estrogen bioassay, *Proc. Soc. Exper. Biol. & Med.* **73:** 672–676 (Apr.) 1950.
4. MAGALLON, D. T., and MASTERS, W. H.: Basal temperature studies in the aged female: influence of estrogen, progesterone, and androgen, *J. Clin. Endocrinol.* **10:** 511–518 (May) 1950.
5. MASTERS, W. H., and MAGALLON, D. T.: Androgen administration in the postmenopausal woman, *J. Clin. Endocrinol.* **10:** 348–358 (March) 1950.
6. MEIER, R.; GASCHE, P., and FREY, H.: Wirkung des Nebennierenrindenhormons als Kristallsuspension, *Schweiz. med. Wchnschr.* **76:** 107–120 (Feb.) 1946.
7. RICHARDS, R. K.; SPRUTH, H. C., and RUSSELL, M. K.: Prolongation of estrus by injection of suspensions of estrogen crystals, *Proc. Soc. Exper. Biol. & Med.* **48:** 400–403 (Nov.) 1941.
8. MEISCHER, K.; GASCHE, P., and FREY, H.: Depotwirkung von Kristallsuspensionen weiblicher sexual Hormone (Ovocylin und Lutocylin-Kristallampullen). Experimentelle und theoretische Grundlagen, *Helvet. physiol. acta* **2:** 515–532, 1944.
9. KOREF, O., and ENGEL, P.: Administration of progesterone in the form of microcrystals, *Endocrinology* **38:** 214–215 (March) 1946.
10. CHANG, M. C.: Maintenance of pregnancy in intact rabbits in the absence of corpora lutea, *Endocrinology* **48:** 17–24 (Jan.) 1951.
11. FREED, S. C., and GREENHILL, J. P.: Therapeutic use of estrone suspensions, *J. Clin. Endocrinol.* **1:** 983–985 (Dec.) 1941.
12. JOEL, C. A.: Zur Therapie der klimakterischen Ausfallerscheinungen mit Oestradiol —Monobenzoat—Kristallen, *Schweiz. med. Wchnschr.* **79:** 1132–1133 (Nov.) 1949.
13. LONG, R. C., and BRADBURY, J. T.: Induction and maintenance of decidual changes with progesterone and estrogen. *J. Clin. Endocrinol.* **11:** 134–145 (Feb.) 1951.

PARAHYDROXYPROPIOPHENONE EFFECTS ON RAT THYROID, ADRENAL, AND PITUITARY*

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THE suggestion, based on clinical observation, that parahydroxypropiophenone[1] is an effective inhibitor of thyrotropic hormone (1, 2) has not to date received any experimental support (3, 4). The chief evidence against such an action is the fact that the goitrogenic effect of propylthiouracil, known to be mediated through the anterior pituitary, is not cancelled by this agent. Our own studies herein presented are in agreement on this point but differ from those cited, in that the administration of parahydroxypropiophenone alone was associated with distinct changes in the thyroid, the adrenal, and the pituitary.

EXPERIMENTAL PROCEDURE

Growing female rats of the Wistar strain, approximately 42 days old, were maintained on ground pellets with and without the addition of parahydroxypropiophenone or propylthiouracil, alone or in combination, as noted in Table 1. At the termination of each experiment serum was obtained for protein-bound iodine analyses (5, 6) and weights of the thyroid, adrenal, and pituitary glands were determined by means of a Roller balance, following meticulous removal of adventitious tissues. These organs were then sectioned for histologic study.

RESULTS

1. *Changes in body weight, in serum protein-bound iodine concentration, and in gland weights*

From Table 1 it is readily evident that ingestion of parahydroxypropiophenone as a 2 per cent admixture in the diet interfered with the gain in body weight of immature rats. This inhibition was not as great, however,

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[1] We are indebted to the Chilcott Laboratories for supplies of parahydroxypropiophenone.

TABLE 1. WEIGHT OF THYROID, ADRENAL, AND PITUITARY GLANDS (MEAN ± S.D. IN MG.*) WITHOUT AND WITH ADDITION OF PARAHYDROXYPROPIOPHENONE OR PROPYLTHIOURACIL TO DIET, ALONE OR TOGETHER, FOR 42 AND 48 DAYS, RESPECTIVELY

	No. of rats	Body wt. (Gm.)	Thyroid wt.		Adrenal wt.		Pituitary wt.		PBI γ% †
			Absolute	% Body wt.	Absolute	% Body wt.	Absolute	% Body wt.	
				(×1,000)		(×1,000)		(×1,000)	
EXPERIMENT I									
Control	19	179.8±14.4	13.3± 1.6	7.5± 1.2	51.2±8.7	28.4±1.3	13.4±2.3	7.4±1.3	3.4
p-OH-propiophenone (2.0%)	12	156.0± 7.2‡	31.6± 5.8‡	19.7± 4.1‡	34.8±6.0‡	22.2±3.9‡	12.8±1.5	8.3±1.0	2.4
Propyl-S-uracil (0.5%)	20	122.6±11.5‡	34.4±11.5‡	27.9± 7.9‡	20.6±4.1‡	16.9±3.0‡	10.0±1.8‡	8.2±1.5	2.5
p-OH-propiophenone (2.0%) + Propyl-S-uracil (0.5%)	19	126.1±10.0‡	37.8± 8.8‡	29.4± 7.5‡	22.9±4.2‡	18.0±3.4‡	10.7±1.6‡	8.3±1.4‡	2.8
EXPERIMENT II									
Control	21	171.0±14.2	13.8± 1.9	8.0± 1.3	39.0±6.3	23.2±3.2	8.3±2.4	4.8±1.3	3.6
p-OH-propiophenone (2.0%)	17	136.5±18.0‡	15.8± 4.3	10.7± 2.2‡	29.3±5.3‡	20.8±3.3‡	6.2±2.5‡	4.4±1.8	3.6
Propyl-S-uracil (0.5%)	17	101.0± 9.8‡	50.4±11.8‡	55.5±17.4‡	21.0±3.7‡	20.7±3.4‡	7.2±2.8	7.1±2.7‡	2.3
p-OH-propiophenone (2.0%) + Propyl-S-uracil (0.5%)	19	101.8±10.3‡	47.5± 9.1‡	46.6± 7.1‡	20.9±3.0‡	20.4±2.8‡	8.2±1.7	8.0±1.9‡	1.9

* As absolute values, as well as in relation to body weight.
† After pooling of sera into four or five groups each consisting of 2 to 4 animals, hence not expressed statistically.
‡ Significantly greater or less than controls ($p = <0.05$).

as that observed on a diet containing 0.5 per cent of propylthiouracil. Moreover, a combined dietary intake of these two compounds in the proportions described did not further suppress growth nor cancel the effects observed with propylthiouracil alone.

Protein-bound iodine levels were lowered in propiophenone-treated animals in the first experiment but not in the second. Propylthiouracil therapy decreased the protein-bound iodine levels in both groups.

When given alone, parahydroxypropiophenone definitely increased thyroid weight in the first experiment; in the second study this change was manifest only in relation to the body weight. Ingestion of parahydroxypropiophenone did not modify the goitrogenic effect of propylthiouracil. In each experiment administration of parahydroxypropiophenone was associated with a decrease in adrenal gland weight, in absolute terms as well as in excess of the lowered body weight. This effect was, in general, not as pronounced as that observed in rats treated with propylthiouracil alone or in combination with parahydroxypropiophenone. In contrast to the consistent adrenal changes, pituitary weight was only sporadically affected, irrespective of the compound or compounds administered.

2. *Histology of thyroid, adrenal, and pituitary glands*

The photomicrographs in Figure 1 represent thyroid findings in each series of rats in the first experiment and indicate that: a) propiophenone produces hyperplasia similar to that which follows propylthiouracil ingestion, and b) this hyperplasia is not discernibly modified by administering the two agents in combination. The goitrogenic effects of the propiophenone in the second experiment, in keeping with the minimal gland weight changes cited earlier, were less marked (Fig. 2B). On the other hand, the thyroids of animals receiving propylthiouracil alone or with propiophenone were indistinguishable from those observed in comparable groups of rats in the first experiment.

The changes in adrenal gland size in parahydroxypropiophenone- or in propylthiouracil-treated rats are attributable in both experiments to a decrease in the adrenal cortex without detectable alteration in the medulla.

Ingestion of propylthiouracil with or without the propiophenone derivative was associated with an increase in the basophilic cells of the anterior pituitary, vacuolization, and hyaline droplet formation. There was an accompanying decrease in the number of recognizable granular acidophilic cells. Similar but less intense changes were seen in propiophenone-treated rats. On the other hand, maintenance on the stock diet did not alter the histologic pattern of the anterior pituitary.

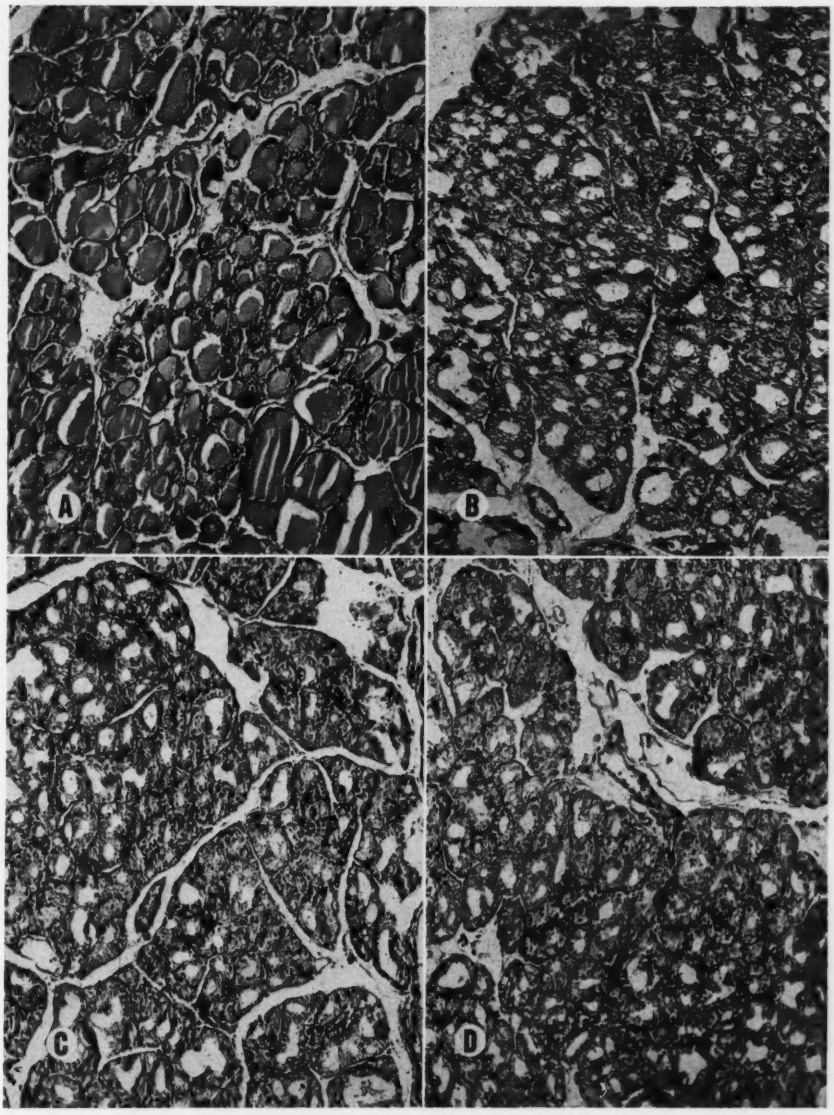

FIG. 1. Photomicrographs (×135) of thyroid glands in Experiment I. A: gland of rat in control series; B: thyroid hyperplasia in rat receiving parahydroxypropiophenone; C: hyperplasia in propylthiouracil-treated animal; and D: hyperplastic thyroid from rat receiving both parahydroxypropiophenone and propylthiouracil.

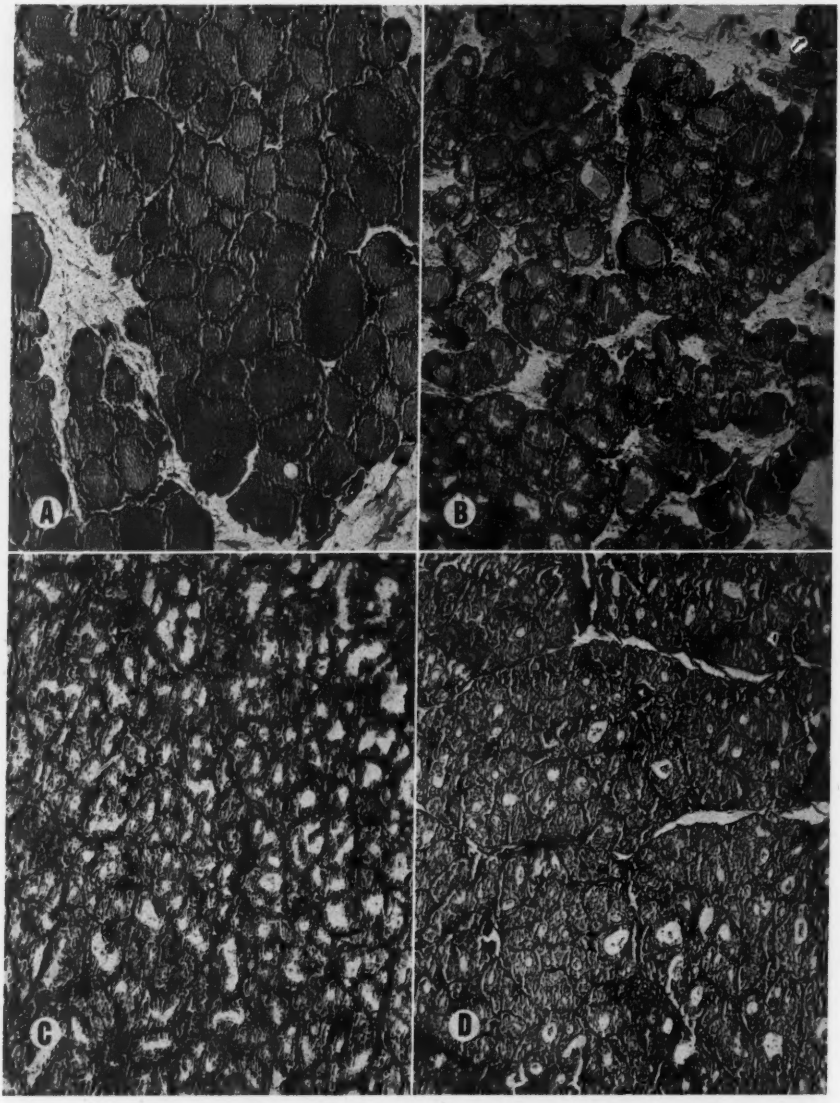

FIG. 2. Photomicrographs (×135) of rat thyroids. Slight hyperplasia developed in the glands of rats in Experiment II receiving parahydroxypropiophenone alone (B), whereas pronounced hyperplasia was observed with propylthiouracil alone (C), and with the two compounds in combination (D). Figure 2A is a representative section from the thyroid gland of an untreated animal.

DISCUSSION

These experiments support the reported finding that parahydroxypropiophenone does not cancel the goitrogenic effect of propylthiouracil. The first drug, however, did induce changes in thyroid and adrenal weight as well as in growth, which qualitatively resembled those seen with propylthiouracil administration at lower levels in the diet. This does not, of course, establish a common mechanism of action, but is compatible with such a possibility. The changes in the thyroid gland could then represent hypertrophy of the type seen with increased TSH resulting from diminished thyroid activity. The latter has been shown in turn to result in decreased adrenal gland weight (7–10) but, under some experimental conditions, comparable changes are observed with inanition *per se* (11). The treated rats in our series did not eat as well as the controls. It should be pointed out also that interference with pituitary ACTH could account for the target-gland changes. Our data do not establish which of these factors, if any, was responsible for the decrease in adrenal weight.

The changes in the basophils of the anterior pituitary observed in the rats receiving propylthiouracil alone or with propiophenone were of the type which follows thyroidectomy or suppression of thyroid function by other means (12, 13). The less marked pituitary change seen in the rats with propiophenone-induced thyroid hyperplasia could be a manifestation of pituitary effect of the compound or, more likely, merely an indication of a lesser degree of thyroid inhibition.

The results of our studies are in part at variance with the reports of Money *et al.* and of Schaffenburg and his colleagues, indicating that thyroid gland weight was either unchanged or actually fell, while the adrenal weights were unaltered or possibly increased (3, 4). It should be pointed out, however, that the experimental conditions were not comparable with respect to the strain, sex, and age of the rats, the dosage of the drug, and the route and duration of therapy.

SUMMARY AND CONCLUSIONS

1. Dietary admixture of parahydroxypropiophenone at a level of 2 per cent interfered with the growth of immature male Wistar rats, increased the mean thyroid weight in absolute and relative terms in one experiment and in relation to the lowered body weight in another, and decreased the mean weight of the adrenals. In general, these changes were less marked than those observed in animals maintained on 0.5 per cent propylthiouracil.

2. Parahydroxypropiophenone produced hyperplasia of the thyroid gland, histologically indistinguishable from that which follows propylthiouracil therapy. This was associated with "thyroidectomy cells" in the pituitary, of the type seen in greater numbers in all of the animals receiv-

ing propylthiouracil. The foregoing findings suggest that under the conditions of these experiments parahydroxypropiophenone acted as a goitrogen.

3. Parahydroxypropiophenone did not alter the changes in rat thyroid, adrenal, and pituitary produced by propylthiouracil therapy.

REFERENCES

1. PERRAULT, M.; VIGNALOU, J., and ETIENNE, M.: Chorionéphithéliome malin (metastases pulmonaires actives) traité avec des résultats satisfaisants, cliniques, hormonaux et radiologiques, par le seul emploi d'un "frénateur hypophysaire de synthèse," la paraoxypropiophénone (corps H 365) pendant dix mois, *Bull. et mém. Soc. méd. d. hôp. de Paris* **65:** 1008–1013 (July) 1949.
2. Paraoxypropiophenone, a powerful synthetic hypophysial inhibitor, (Foreign Letter) *J.A.M.A.* **145:** 341–342 (Feb. 3) 1951.
3. MONEY, W. L.; LUCAS, V.; FAGER, J., and RAWSON, R. W.: The effects of *p*-hydroxypropiophenone on the thyroid gland of experimental animals, *J. Clin. Endocrinol.* **11:** 973–977 (Sept.) 1951.
4. SCHAFFENBURG, C. A.; MASSON, G. M.; MCCULLAGH, E. P., and HAOUR, P.: *p*-Hydroxypropiophenone (PHP) and other so-called pituitary inhibitors, *J. Clin. Endocrinol.* **11:** 1215–1223 (Nov.) 1951.
5. BARKER, S. B.: Determination of protein-bound iodine, *J. Biol. Chem.* **173:** 715–724 (Apr.) 1948.
6. DANOWSKI, T. S.; JOHNSTON, S. Y., and GREENMAN, J. H.: Alterations in serum iodine fractions induced by the administration of inorganic iodide in massive dosage, *J. Clin. Endocrinol.* **10:** 519–531 (May) 1950.
7. MARINE, D., and BAUMANN, E. J.: Hypertrophy of adrenal medulla of white rats in chronic thiouracil poisoning, *Am. J. Physiol.* **144:** 69–73 (June) 1945.
8. BAUMANN, E. J., and MARINE, D.: Involution of the adrenal cortex in rats fed with thiouracil, *Endocrinology* **36:** 400–405 (June) 1945.
9. ZARROW, M. X., and ZARROW, I. G.: Mechanism of adrenal involution in the rat after treatment with thiouracil, *Proc. Soc. Exper. Biol. & Med.* **76:** 620–623 (Apr.) 1951.
10. DALTON, A. J., and MORRIS, H. P.: Changes in the organs of female C3H mice receiving thiourea, *J. Nat. Cancer Inst.* **5:** 451–454 (June) 1945.
11. MULINOS, M. G., and POMERANTZ, L.: Hormonal influences on the weight of the adrenal in inanition, *Am. J. Physiol.* **132:** 368–374 (Mar.) 1941.
12. GRIESBACH, W. E., and PURVES, H. D.: The significance of the basophil changes in the pituitary accompanying various forms of thyroxine deficiency, *Brit. J. Exper. Path.* **26:** 13–17 (Feb.) 1945.
13. GOLDBERG, R. C., and CHAIKOFF, I. L.: The cytological changes that occur in the anterior pituitary glands of rats injected with various doses of I^{131} and their significance in the estimation of thyroid function, *Endocrinology* **46:** 91–104 (Jan.) 1950.

THE METABOLISM OF RADIOTHYROXINE IN EXOPHTHALMIC GOITER*†

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INTRODUCTION

THE preparation of thyroxine labeled with radioiodine by Joliot and co-workers in 1944 (1) has led to numerous studies of the behavior of this substance in animals (2–10), but in the human it has been investigated only recently. Albert and co-workers (11, 12) studied the distribution and fate of *d-l*-radiothyroxine in myxedematous patients after oral and intravenous administration. Myant and Pochin (13) made similar observations in normal, or euthyroid subjects, and mentioned briefly some results in a patient with exophthalmic goiter. The purpose of this paper is to report the results of tracer studies with radioactive thyroxine in 6 patients with thyrotoxicosis.

MATERIALS AND METHODS

Six patients with clinically active exophthalmic goiter volunteered as subjects of this study. None of the patients had received antithyroid drugs for one month, or iodine for eleven days, prior to the study. Pertinent data for each patient are give in Table 1.

Labeled thyroxine of high specific activity (1.5 to 6.5 millicuries per milligram) was prepared by exchange (Abbott Laboratories, North Chicago, Illinois). The radiothyroxine given the first patient was racemic, but that given the others was levorotatory. A 500-microcurie portion of radiothyroxine was diluted to a volume of 10 ml. with sterile distilled water

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at 39° C. Six milliliters of this solution, containing approximately 300 microcuries of I^{131}, was injected into an antecubital vein of the patient within one hour after dilution. One milliliter of the same dilution was introduced into a volumetric flask, together with water and stable thyroxine carrier, to serve as a standard solution. For the intravenous iodide tracer, which was injected as a control in Patient 6, a solution of 500 microcuries of sodium radioiodide (103 microcuries per microgram of NaI) in 10 ml. of water was sterilized. Six milliliters was injected intravenously and 1 ml. was introduced into a volumetric flask with carrier to serve as a standard solution.

TABLE 1. CLINICAL STATUS OF THE 6 PATIENTS

Patient	Age, years	Sex	B.M.R., %	P.B.I., μg. %	Remarks
1	40	F	+20		Recurrence of moderate toxicity, 2-yr. duration; 1 previous thyroidectomy
2	54	M	+45		Mild, 10-mo. duration
3	32	F	+ 9		Recurrence of moderate toxicity, 3-yr. duration; 2 previous thyroidectomies
4	56	F	+39	8.3	Moderate, 6-mo. duration
5	57	F	+67	13.1	Severe, 2-wk. duration
6	48	F	+13	11.6	Recurrence of moderate toxicity, 1-yr. duration; 1 previous thyroidectomy

Immediately following injection of labeled thyroxine and at frequent intervals for the first eight hours, *in vivo* measurements were made at 10 cm. over the liver, left epigastrium, thyroid and thigh with a Victoreen Geiger-Müller counter and an appropriate scaling circuit. A 2-mm. lead filter was used and counts were made for a duration of 100 to 200 seconds, using a modification of the method described by Keating and co-workers (14). *In vivo* counts were repeated daily after the first eight hours for four to six days.

Venous blood was withdrawn at twenty and forty minutes, at one, two, four, eight and twelve hours, and thereafter daily. In the last 4 patients an additional specimen was obtained five minutes after injection. Urine was collected at two-hour intervals for the first twelve hours and thereafter in twelve-hour periods for the duration of the experiment (15). All stools were collected as passed and the time was recorded.

At the completion of the thyroxine study all patients were given a tracer dose of sodium radioiodide, administered orally to the first 5 subjects and

intravenously to the sixth subject. Iodide tracer results were analyzed by the methods of Keating and co-workers (15).

The fractionation procedure of Albert and Keating (16) was used to determine the relative amounts of thyroxine, diiodotyrosine and iodide in each specimen of blood and urine. The efficiency of the fractionation procedure was such that 90 per cent or more of each added radioactive compound was recovered in control experiments (16). The presence of other, unidentified compounds of radioiodine in these fractions is not excluded.

The counting and concentration methods were those previously described by Rall and co-workers (17). Urine specimens containing more than 0.1 microcurie in 10 ml., and aliquots of feces, were counted in a well-type gamma ray counter (5).

RESULTS

Because of the limitation of space, detailed data will be presented for only Patient 6. Comparisons with the other patients are summarized in Table 2. Radioactivity in the thyroid, urine, feces and serum of Patient 6

TABLE 2. SUMMARY OF TRACER DATA

Patient	Thyroxine tracer						Iodide tracer				
	I^{131}, $\mu c.$	Thyroxine, $\mu g.$	$Qt_{(1)}$	$Qu_{(2)}$	$Tu_{(4)}$	$Tf_{(4)}$	$Qt_{(1)}$	$Qu_{(2)}$	AR	RER	ERDR
1	304	187	32.4	17.0	18.2	9.1	60.5	29.8	20.4	9.5	24.2
2	311	187	12.6	39.1	45.2	11.3	38.6	65.3	3.4	6.1	2.9
3	300	137	11.7	18.1	24.3	4.8	39.8	58.8	5.6	7.9	6.2
4	300	151	17.0	7.9	11.3	7.2	52.4	29.5	10.6	5.2	15.0
5	300	47	21.9	11.9	15.6	11.2	65.2	22.1	22.2	6.0	28.0
6	300	63	23.7	22.1	26.4	12.5	51.4	37.1	31.1	17.0	43.6

The dose of the iodide tracer was 100 $\mu c.$ of I^{131} + 100 $\mu g.$ of I^{127} orally in each case except No. 6, in which it was 300 $\mu g.$ of I^{131} + 3.0 $\mu g.$ of I^{127} intravenously.

Abbreviations: $Qt_{(1)}$ = Thyroid fraction at 24 hrs.
$Qu_{(2)}$ = Urinary fraction at 48 hrs.
$Tu_{(4)}$ = Total I^{131} in urine at 96 hrs.
$Tf_{(4)}$ = Total I^{131} in feces at 96 hrs.
AR = Accumulation rate
RER = Renal excretion rate
ERDR = Extrarenal disposal rate

following the administration of radiothyroxine is shown in Figure 1. The curve for serum I^{131} was composed of two main phases, an initial rapid decrease for about the first twelve hours or so, followed by a very slow decline for the duration of observations. The shapes of the curves for thyroidal, urinary, blood and fecal I^{131} were substantially similar from patient to patient, but the relative quantities involved varied. Thus, in Patients 1, 3, 4 and 6 (*see* also Table 2), the relative position of the four curves was

as shown in Figure 1, with the thyroid containing more radioiodine than the urine and the urine more than the feces. In Patients 2 and 5, however, the urine contained more than the thyroid. Such differences probably represent differences in the intensity of thyroid disease from patient to patient.

The shape of the curves following the administration of the radioiodide tracer (Fig. 2) has been described in detail elsewhere (18, 19). These are

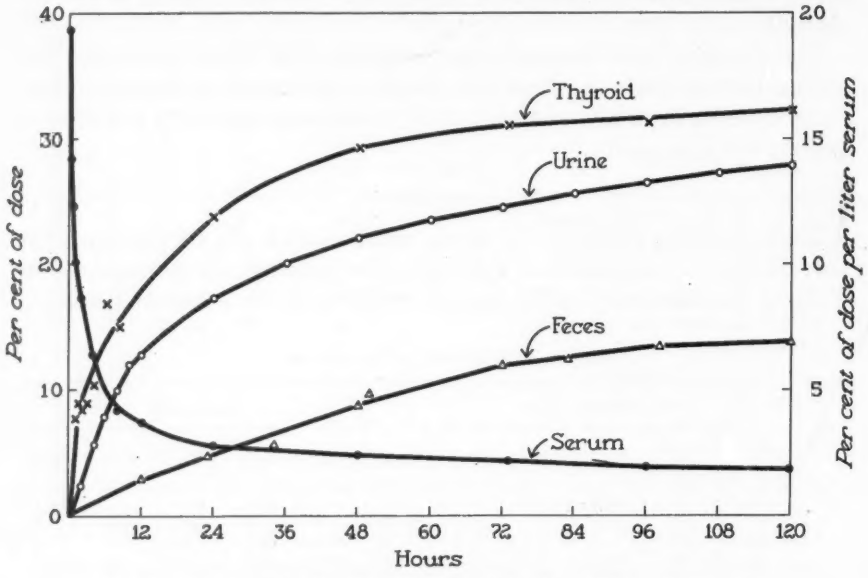

FIG. 1. Changes of radioactivity in thyroid, urine, feces and serum of Patient 6 after administration of radiothyroxine.

essentially exponential curves with rapid rates. The difference between these curves and those after administration of the radiothyroxine tracer (Fig. 1) is that the former are relatively simple exponential functions, whereas the latter are not.

A. Radiothyroxine tracer

The mean half-value time for the initial rapid phase of serum I^{131} in the 6 patients, was 3.3 ± 0.56 hours, and for the late phase, 135 ± 12 hours. The significance of the difference in rates between these two components will be discussed later. The data on the separation of the I^{131} of serum into three components, comprising thyroxine, diiodotyrosine, and iodide, are given in Table 3. For the entire period of observations the major component of the serum I^{131} was radiothyroxine. The values for diiodotyrosine

were negligible, being the same as, or less than, the error of the technic. However, small amounts of iodide were found at varying times during the experiment. In general, the results of the fractionation data on the remaining 5 patients were similar. At no time were there significant amounts of I^{131} present as diiodotyrosine and in all cases the percentage of thyroxine I^{131} was between 75 and 95 per cent, and that of iodide between 0 and 15 per cent of the total I^{131}.

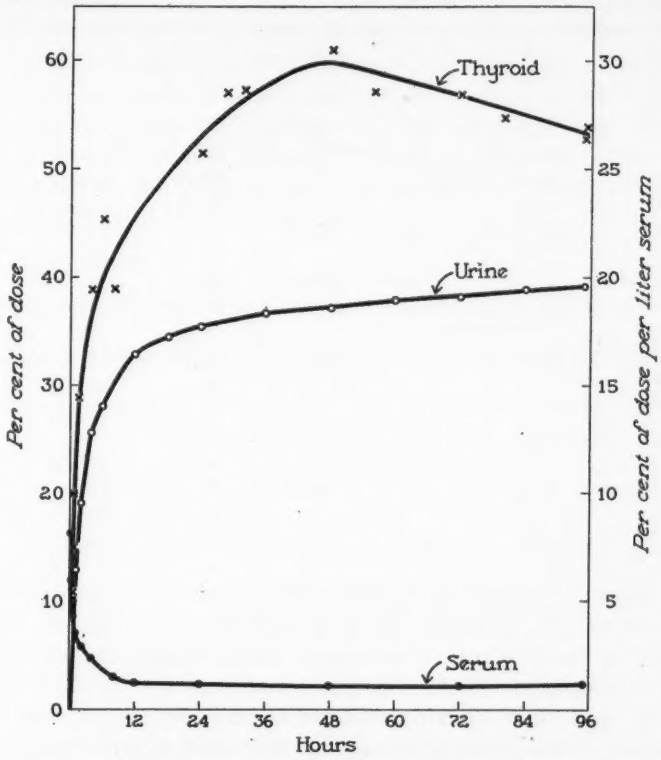

FIG. 2. Changes of radioactivity in thyroid, urine and serum of Patient 6 after administration of radioiodide.

The accumulation of I^{131} in the thyroid began almost immediately after injection, and continued for the first two days. After forty-eight hours, little further collection occurred. The proportion of the dose present in the thyroid at one and at four days is shown in Table 2. In general, the proportion of I^{131} collected following administration of a radiothyroxine tracer was about one third of that collected following administration of an iodide tracer.

TABLE 3. FRACTIONATION DATA ON PATIENT 6 AFTER ADMINISTRATION OF THYROXINE TRACER

Time, hours	Serum			Urine		
	Per cent I^{131} present as:			Per cent I^{131} present as:		
	Iodide	Diiodotyrosine	Thyroxine	Iodide	Diiodotyrosine	Thyroxine
0.08	9.1	6.4	84.6			
0.33	10.0	5.5	84.5			
0.58	9.3	5.3	85.4			
1	9.3	4.5	86.2			
2	9.1	3.9	87.0	51.9	27.5	20.7
4	18.0	4.1	77.4	65.0	14.7	20.3
6				70.4	15.6	14.0
8	10.3	4.4	85.3	68.0	19.8	12.1
10				71.9	17.8	10.3
12	10.0	2.9	87.0	80.9	9.7	9.4
24	6.6	2.2	91.2	78.0	11.3	10.7
36				81.4	7.8	10.8
48	13.7	2.0	84.3	73.8	15.8	10.4
60				70.5	20.3	9.3
72				76.2	12.5	11.2
73	8.2	2.1	89.6			
84				76.8	12.0	11.2
96	11.5	2.2	86.3	75.5	15.0	9.5
108				75.7	15.5	8.8
120	18.2	2.0	79.8	75.5	14.3	10.2

The urinary excretion curves were similar in shape to the thyroidal collection curves after administration of radiothyroxine tracers. Excretion was rapid for the first twelve hours and then gradually diminished. Table 2 shows a comparison of the 6 patients with regard to urinary I^{131} at the end of two days. Radioiodide continued to be excreted at a slow constant rate in most of the patients throughout the entire experimental period of 120 or more hours. The chemical form of the I^{131} in the urine of Patient 6 is indicated in Table 3. Although some radiothyroxine may have been present in urine during the first few hours following injection of radiothyroxine, the proportion of thyroxine-like I^{131} to the total I^{131} in the urine was very small. The same situation seemed to be true also for diiodotyrosine-like I^{131}. The proportion of iodide, however, increased gradually until about four fifths of the urinary I^{131} was present as iodide. With minor variations, this same situation held for the remaining 5 patients. During the first six hours, the proportion of radiothyroxine was between 0 and 20 per cent of total urinary radioactivity but rapidly fell to insignificant levels

by the end of the first day. The percentage of diiodotyrosine varied between 5 and 20 per cent of the total; and the proportion of iodide, the main urinary constituent, varied among the 5 patients from 50 to 90 per cent of the total urinary I^{131}.

Excretion of I^{131} in the feces paralleled that in the urine, and was always less in quantity than urinary excretion. Fecal excretion of I^{131} began twelve or more hours after urinary excretion of I^{131}.

In vivo measurements over the liver and the left epigastrium were maximal at an average time of between one and one and a half hours after injection of radiothyroxine. The counting rate over the liver averaged thirteen times the rate over the thigh, and the rate over the left epigastrium, nine times the rate over the thigh at one hour after injection. The rates over the liver and the left epigastrium then diminished fairly rapidly until twelve hours. After twelve hours there was a further, slower diminution which appeared to be exponential.

B. Radioiodide tracer

Following administration of inorganic radioiodide, I^{131} in the serum

TABLE 4. FRACTIONATION DATA ON PATIENT 6 AFTER ADMINISTRATION OF IODIDE TRACER

Time, hours	Serum			Urine		
	Per cent I^{131} present as:			Per cent I^{131} present as:		
	Iodide	Diiodotyrosine	Thyroxine	Iodide	Diiodotyrosine	Thyroxine
0.08	74.0	15.4	10.6			
0.25	75.4	11.5	13.1			
0.58	74.0	6.8	19.2			
1	69.5	8.2	22.3	98.7	0.9	0.4
2	59.9	8.5	31.6	97.9	1.2	0.9
4	55.6	8.9	35.4	95.2	3.9	1.0
6				95.8	3.0	1.3
8	38.9	6.5	54.6			
12	28.8	4.0	67.2	91.0	7.8	1.3
18				87.3	10.3	2.4
24	21.9	4.8	73.3	69.0	27.7	3.3
36				69.7	25.7	4.7
48	16.5	2.3	81.2	45.0	27.5	27.5
60				52.2	35.0	12.8
72	20.9	3.6	75.5	37.7	29.9	32.4
84				50.0	27.9	22.1
95		3.0	97.6	23.0	26.0	51.0

decreased rapidly for about twelve hours and then remained at a fairly steady level (Fig. 2). The nature of this curve, however, is quite different from that following administration of radiothyroxine, since the initial phase resembles a simple exponential curve (19) and the secondary slower phase represents at least two components: replacement of the serum radioiodide with organic I^{131}, and disappearance of radioiodide. The changing chemical nature of the serum I^{131} content is given in Table 4, where it is evident that a progressive increase occurred in the percentage of thyroxine I^{131}. By the ninety-fifth hour, 98 per cent of the I^{131} in the serum was present as thyroxine. Insignificant amounts of diiodotyrosine-like I^{131} were detected.

The thyroidal accumulation of radioactivity rapidly reached a peak at forty-eight hours, after which a progressive decrease occurred. The curve of thyroidal I^{131} is typical for patients with thyrotoxicosis. Excretion of I^{131} in the urine was virtually complete after the first day, very little increment occurring thereafter. For the first twelve hours, 90 per cent or more of the urinary radioiodine was present as iodide and insignificant amounts as diiodotyrosine or thyroxine. Thereafter a progressive increase in the percentage present as thyroxine and diiodotyrosine occurred, so that by the end of the period of observation, only about 20 per cent of the urinary I^{131} was present as iodide. This changing chemical nature of the urinary I^{131} was proportionate to the changes described in blood.

DISCUSSION

Behavior of I^{131} in serum. The curve of radioactivity in the serum after administration of radiothyroxine appears to consist of multiple regressions which are not readily determined with the limited data here obtained. However, if the curve of serum total radioactivity be extrapolated to zero time, an estimate can be made of the I^{131} in serum under conditions of instantaneous mixing. The dose divided by this extrapolated value should approximate the serum volume. Such estimates are compared in Table 5 with estimations of serum volume based on body weight. The calculations based on radioactivity suffer from the fact that the first few blood values,

TABLE 5. COMPARISON OF SERUM VOLUMES (LITERS), ON BASIS OF RADIOACTIVITY MEASUREMENTS AND OF BODY WEIGHT

	Patient No.					
	1	2	3	4	5	6
Radioactivity	12.0	8.6	4.2	7.4	2.1	2.9
Body weight	2.2	3.5	4.7	2.7	2.1	2.9

which determine the extrapolation of the curve, were not obtained soon enough after injection of radiothyroxine in all patients, and especially in Patients 1 and 2. In spite of this shortcoming, the comparison indicates that a significant fraction of radiothyroxine must have left the blood within twenty minutes after injection.

The fall of the blood I^{131} concentration during the first twelve hours was extremely rapid. This initial portion of the blood curve does not fit a simple exponential function, no matter whether the total or thyroxine-like I^{131} values were used, and presumably represents several events occurring simultaneously. Thus, radiothyroxine must be disappearing from the serum not only into its diffusion space, but also into certain organs. The localization of radioactivity over the liver area in these patients, observed by *in vivo* counting, suggests that radiothyroxine may be concentrated in the liver and gastro-intestinal tract. This supposition receives support from animal work (20) in which about one half of the dose of radiothyroxine was found in the liver of rats one minute after intravenous injection.

The slow decline in concentration which constitutes the second portion of the curve of serum I^{131} may be presumed to represent: 1) the rate of breakdown of thyroxine, 2) the excretion of it or of its metabolites from the body, and 3) the rate at which the thyroid renews the circulating organic I^{131}. By extrapolation of this phase to time zero on a semilogarithmic plot a diffusion volume of thyroxine may be calculated (assuming the slow phase of the curve to be exponential). In some of the patients this volume exceeded body weight, a result which can be explained only by assuming a concentration of radiothyroxine somewhere in the body greater than its concentration in the serum.

It was possible for Albert and Keating (12) to calculate volumes of distribution for radiothyroxine in a patient with myxedema, using rate constants determined from the serum concentration curve. Myant and Pochin (13) calculated similar volumes for normal subjects by subtracting urinary excretion and thyroid content from the dose and dividing this difference by the plasma concentration. Since in the present study it was not possible to determine and utilize rate constants, spaces were calculated by a modification of the method of Myant and Pochin (13): $V_D = 100 - (T + U + F) / S_T$ where T, U and F are the percentages of the dose in the thyroid, urine and feces, respectively, and S_T is the serum concentration of I^{131} in percentage of the dose per liter. When V_D was plotted versus time, a curve was obtained with a rapid rise in the first twelve hours. After twelve hours, a gradual rise was noted throughout the duration of observations in the first 4 patients, a constant level in the fifth patient and a gradual fall in the sixth patient. The rapid rise would seem to represent diffusion into the thyroxine space, and the second slow phase may represent concentration

and retention in body tissues. By extrapolating the slow, second phase to zero time, it was possible to estimate thyroxine space under conditions of instantaneous diffusion. Expressed in percentage of body weight, the average space in the 6 subjects was 34.1 ± 4.2 per cent. This agrees essentially with an estimate of 32.6 per cent of body weight in normal subjects by Myant and Pochin (13), but is higher than an estimate of 19.2 per cent of body weight by Albert and Keating (12) for their patient with myxedema.

Clearances. Renal clearance calculations were made for each of the patients, using the standard formula: $Cl = U/\overline{P}(t_2 - t_1)$ where U is the content of thyroxine in the urine collected from time 1 to time 2 and $\overline{P}$ is a value for serum concentration estimated from the curve of serum thyroxine

TABLE 6. THE PROPORTION OF THYROIDAL RADIOIODINE IN 6 PATIENTS AFTER ADMINISTRATION OF RADIOTHYROXINE AND RADIOIODIDE TRACERS

	Patient No.					
	1	2	3	4	5	6
Radiothyroxine	0.78	0.41	0.50	0.80	0.79	0.66
Radioiodide	0.68	0.39	0.43	0.66	0.77	0.61

concentration at the mean time between t_1 and t_2. When clearances so obtained were plotted against time, they seemed to decrease with time. This may possibly be due to the increasing inaccuracies in the estimation of urinary thyroxine. The average thyroxine clearance between twelve and twenty-four hours was 0.25 ± 0.08 ml. per minute. Myant and Pochin (13) estimated renal clearance of thyroxine at 0.1 ml. per minute in their 6 normal subjects, whereas renal thyroxine clearance for the patient with myxedema (12) by the method of Newman, Gilam and Philips (21) was 0.8 ml. per minute.

Thyroidal uptake. The accumulation of I^{131} by the thyroid after the administration of radiothyroxine presumably results from radioiodide released during the catabolism of radiothyroxine. That the thyroid collects radioiodide rather than radiothyroxine or some radioactive organic derivative of thyroxine, was indicated by Myant and Pochin (13). These authors showed that the ratio of the thyroidal I^{131} to urinary radioiodide was essentially the same after administration of either radiothyroxine or radioiodide tracers. This similarity is best explained by assuming that radioiodide alone enters the gland. Similar calculations on the patients reported herein are given in Table 6, where the ratios were calculated as the percentage of the dose in the thyroid divided by the sum of the percentage of the dose in the thyroid and the percentage of the dose in the urine as radioiodide. The ratios for each of the subjects are sufficiently similar to

indicate that the proposition advanced by Myant and Pochin for euthyroid subjects (that radioiodide alone enters the thyroid gland) applies also to thyrotoxic subjects.

Release of iodide. If the proposition that the thyroid collects only radioiodide is correct, it is possible, as was done in euthyroid cases (13), to estimate the amount of iodide released from thyroxine. The thyroidal I^{131} and the urinary radioiodide represent two large, easily determined components of iodide liberated from radiothyroxine. A third major component of radioiodide is the amount of radioiodide in the iodide space, which was not determined for technical reasons. A rough approximation of the amount of iodide released from thyroxine can be achieved by the simple addition of the first two of these components; this will be in error by the amount in the iodide space. Since the latter becomes smaller with time, some idea of the true value may be obtained from successive estimates. In terms of percentage of dose released as iodide, the mean value for the 6 patients was 18.5 ± 2.4 per cent at six hours and 30.0 ± 3.1 per cent at twenty-four hours. These values agree with similar calculations made in euthyroid subjects by Myant and Pochin—11.9 per cent at six hours and 30.5 per cent at twenty-four hours. These estimations are in error, especially in the calculation at twenty-four hours, to the extent that some radioiodide was resecreted from the thyroid as radiothyroxine.

SUMMARY

Radiothyroxine was injected intravenously as a single dose in 6 patients with exophthalmic goiter. The initial phase of disappearance from the blood was very rapid with a half-value time of three hours. This phase was a complex function depending upon diffusion into body fluids and a rapid concentration by certain tissues, presumably the liver and the gastrointestinal tract. After twelve or more hours, a slow disappearance with a half-value time of five to six days became apparent, presumably due to utilization of the thyroxine by the tissues and excretion in the urine and feces.

Uptake by the thyroid and excretion in the urine after administration of radiothyroxine was slower than after administration of radioiodide, and appeared to depend mostly on the rate of release of radioiodide from radiothyroxine.

The main metabolic fate of thyroxine, aside from some excretion in the feces and the urine, was de-iodination. Part of the iodide thus released was reaccumulated in the thyroid, and part was excreted in the urine, depending upon the ratio of the thyroidal clearance and the renal clearance. About one third of the injected hormone was metabolized in twenty-four hours.

REFERENCES

1. JOLIOT, F.; COURRIER, R.; HOREAU, A., and SÜE, P.: Sur la préparation d'une hormone contenant des atomes radioactivs, *Compt. rend. Soc. de biol.* **138:** 325–327 (June 10) 1944.
2. FRIEDEN, EARL; LIPSETT, M. B., and WINZLER, R. J.: Methods for labeling thyroxine with radioactive iodine, *Science* n.s. **107:** 353–354 (Apr. 2) 1948.
3. JOLIOT, FRÉDÉRIC; COURRIER, ROBERT; HOREAU, ALAIN, and SÜE, PIERRE: Sur l'obtention de la thyroxine marquée par le radioiode et son comportement dans l'organisme, *Compt. rend. Acad. d. sc.* **218:** 769–771 (May 15) 1944.
4. GROSS, J., and LEBLOND, C. P.: Distribution of a large dose of thyroxine labeled with radioiodine in the organs and tissues of the rat, *J. Biol. Chem.* **171:** 309–320 (Nov.) 1947.
5. JOHNSON, H. W., and ALBERT, A.: The excretion and distribution of I^{131} following administration of physiologic amounts of labeled iodide, diiodotyrosine and thyroxine in the rat, *Endocrinology* **48:** 669–681 (June) 1951.
6. GROSS, J., and LEBLOND, C. P.: Metabolism of the thyroid hormone in the rat as shown by physiologic doses of labeled thyroxine, *J. Biol. Chem.* **184:** 489–500 (June) 1950.
7. CLAYTON, J. C.; FREE, A. A.; PAGE, J. E.; SOMERS, G. F., and WOOLLETT, E. A.: Absorption and excretion of the mono-sodium salt of thyroxine labelled with radioactive iodine, *Biochem. J.* **46:** 598–604, 1950.
8. TAUROG, A.; BRIGGS, F. N., and CHAIKOFF, I. L.: I^{131} labeled L-thyroxine. I. An unidentified excretion product in bile, *J. Biol. Chem.* **191:** 29–34 (July) 1951.
9. TAUROG, ALVIN; BRIGGS, F. N., and CHAIKOFF, I. L.: I^{131}-labeled L-thyroxine. II. Nature of the excretion product in bile, *J. Biol. Chem.* **194:** 655–668 (Feb.) 1952.
10. GROSS, J., and LEBLOND, C. P.: Metabolites of thyroxine, *Proc. Soc. Exper. Biol. & Med.* **76:** 686–689 (Apr.) 1951.
11. ALBERT, A.; RALL, J. E.; KEATING, F. R., JR.; POWER, M. H., and WILLIAMS, M. M. D.: The behavior of labeled thyroglobulin and labeled thyroxine in patients with myxedema, *J. Clin. Endocrinol.* **9:** 1392–1405 (Dec.) 1949.
12. ALBERT, A., and KEATING, F. R., JR.: Metabolic studies with I^{131} labeled thyroid compounds. Comparison of the distribution and fate of radioactive *d-l*-thyroxine after oral and intravenous administration in the human, *J. Clin. Endocrinol.* **9:** 1406–1421 (Dec.) 1949.
13. MYANT, N. B., and POCHIN, E. E.: The metabolism of radiothyroxine in man, *Clin. Sc.* **9:** 421–440, 1950.
14. KEATING, F. R., JR.; WANG, J. C.; LUELLEN, T. J.; WILLIAMS, M. M. D.; POWER, M. H., and MCCONAHEY, W. M.: The measurement of the iodine-accumulating function of the human thyroid gland, *J. Clin. Investigation* **29:** 217–227 (Mar.) 1949.
15. KEATING, F. R., JR.; POWER, M. H.; BERKSON, JOSEPH, and HAINES, S. F.: The urinary excretion of radioiodine in various thyroid states, *J. Clin. Investigation* **26:** 1138–1151 (Nov.) 1947.
16. ALBERT, A., and KEATING, F. R., JR.: Metabolic studies with I^{131} labeled thyroid compounds: distribution and excretion of radiodiiodotyrosine in human beings, *J. Clin. Endocrinol.* **11:** 996–1011 (Sept.) 1951.
17. RALL, J. E.; JOHNSON, H. W.; POWER, M. H., and ALBERT, A.: The determination of radioactive iodine in biologic material, *Proc. Soc. Exper. Biol. & Med.* **75:** 390–392 (Nov.) 1950.

18. KEATING, F. R., JR.; HAINES, S. F.; POWER, M. H., and WILLIAMS, M. M. D.: The radioiodine-accumulating function of the human thyroid gland as a diagnostic test in clinical medicine, *J. Clin. Endocrinol.* **10:** 1425–1464 (Nov.) 1950.
19. KEATING, F. R., JR., and ALBERT, A.: The metabolism of iodine in man as disclosed with the use of radioiodine, *in* Recent Progress in Hormone Research. New York, Academic Press, 1949, vol. 4, pp. 429–481.
20. ALBERT, A.: The enterohepatic circulation of radiothyroxine (abstract) *J. Clin. Endocrinol. & Metab.* **12:** 942 (July) 1952.
21. NEWMAN, E. V.; GILAM, A., and PHILIPS, F. S.: The renal clearance of thiosulfate in man, *Bull. Johns Hopkins Hosp.* **79:** 229–242 (Sept.) 1946.

A COMPARISON OF THE HISTOLOGIC PATTERN OF BENIGN AND MALIGNANT THYROID TUMORS*

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THE relationship between benign and malignant thyroid tumors has been for many years a subject for interesting discussion. The interest has been due largely to a common opinion that many, perhaps most, thyroid carcinomas arise from a benign thyroid tumor which pre-existed for a variable length of time before transforming itself into a cancer. The evidence for such an opinion has been mostly clinical; the pathologic demonstration of the actual transformation or transition of a benign thyroid tumor to a malignant one has not always been so clear or as acceptable.

It is known that in most organs of the body benign tumors have malignant counterparts as far as histologic structure is concerned; therefore, in order to further the pathologic understanding and evaluation of thyroid tumors in general, a comparison and contrast of the histologic growth patterns of benign and malignant thyroid neoplasms seemed indicated. Such a study was carried out to determine, in addition to general information regarding thyroid tumors: 1) whether comparable histologic patterns do occur in benign and malignant thyroid neoplasms; 2) if comparable patterns occur, the relative frequency of the respective patterns in benign and malignant states; 3) the relationship, if any, of the various growth patterns to age and sex; and 4) the value of current histologic classifications of thyroid tumors.

The material utilized to carry out this study consisted of 500 thyroid adenomas and 200 thyroid cancers. In each series the cases were consecutive ones and all specimens studied had been surgically resected. In every instance the gross and microscopic structure of the benign or malignant tumor was reviewed, classified and tabulated along with the age and sex of the patient. In the adenoma group only tumors which were considered

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to be true neoplasms were included; nodules of adenomatous (endemic) goiter were disregarded. The criteria used for the diagnosis of an adenoma were those suggested by Warren (1): encapsulation, homogeneous texture, variation from the structure of the gland outside the capsule, and evidence of compression of adjacent thyroid tissue by the nodule.

BENIGN TUMORS

It is quite apparent that, structurally, benign thyroid adenomas fall readily into two groups: a) *follicular*, in which there is some degree or suggestion of follicular pattern; and b) *papillary*, in which there is formation of papillae. The follicular group may be subdivided into several categories based largely on differentiation and colloid content (2, 3): 1) *embryonal* (trabecular), which are composed of columns of cells with little follicle formation and little or no colloid; 2) *fetal*, which are often called microfollicular because of the miniature follicles with small amounts of colloid; 3) *simple*, which have well developed follicles with a colloid content similar to that in a normal gland; 4) *colloid* (macrofollicular) which contain excessive colloid within greatly distended follicles; and 5) *Hürthle-cell*, which are composed predominantly or entirely of large, acidophilic cells popularly known as Hürthle cells.

The foregoing classification of structural pattern was used in this study because it, or a comparable one, is in general usage for thyroid adenomas and because the distinction between groups and subgroups is readily made histologically. There was relatively little overlapping of groups; uniformity of structure within an individual adenoma, except for changes such as fibrosis and hemorrhage, was quite striking. The results of the tabulation of the 500 benign thyroid adenomas are seen in Table 1. The unclassified

TABLE 1. ADENOMAS

	Number	Per cent of total	Average age of patient at time of removal (yrs.)	Per cent in females
Follicular	433	86.6	43	83
Embryonal	(65)	(13.0)	43	89
Fetal	(262)	(52.3)	45	79
Simple	(61)	(12.2)	39	90
Colloid	(19)	(3.8)	41	84
Hürthle-cell	(26)	(5.2)	44	92
Papillary	24	4.8	46	83
Unclassified	43	8.6	43	81
Total	*500*	*100.0*	*43*	*83*

tumors were those which were cystic, hemorrhagic, or so degenerated that determination of structural growth pattern was impossible. Also included in the unclassified group were 6 adenomas considered to be "toxic," that is, histologically hyperplastic and associated with clinical hyperthyroidism.

There are several interesting points in the tabulation of the adenomas which are worthy of emphasis. The follicular group of tumors is many times more common than the papillary group, although the average age of the patient at the time of removal and the high incidence in females is the same in both. Within the follicular subgroups there is again little difference in the average age or the sex incidence, the only variation being due to the higher frequency of the more immature growth patterns (embryonal and fetal). When the adenomas of various types were tabulated according to the decade in which they were removed, again there was no significant variation between either groups or subgroups.

MALIGNANT TUMORS

Using the same criteria for structural classification, the 200 thyroid carcinomas were tabulated in a comparable manner (Table 2). All carcinomas were included, whether early or advanced, whether of low or high

TABLE 2. CARCINOMAS

	Number	Per cent of total	Average age of patient at time of removal (yrs.)	Per cent in females
Follicular	54	27.0	44	76
Embryonal	(7)	(3.5)	41	90
Fetal	(32)	(16.0)	43	81
Simple	(5)	(2.5)	37	80
Colloid	(0)	(0)	0	0
Hürthle-cell	(10)	(5.0)	54	50
Papillary	100	50.0	41	84
Unclassified	46	23.0	58	74
Total	*200*	*100.0*	*48*	*77*

degree of malignancy; the classification was thus based entirely on structural pattern rather than on the extent of cancer or the degree of malignancy. For obvious reasons the anaplastic and undifferentiated cancers could not be classified; the unclassifiable group was composed of the small-cell and giant-cell carcinomas, together making up a total of 23 per cent of all thyroid cancers. In contrast to the adenomas, uniformity of structure in the carcinomas was often lacking; mixtures of papillary and follicular

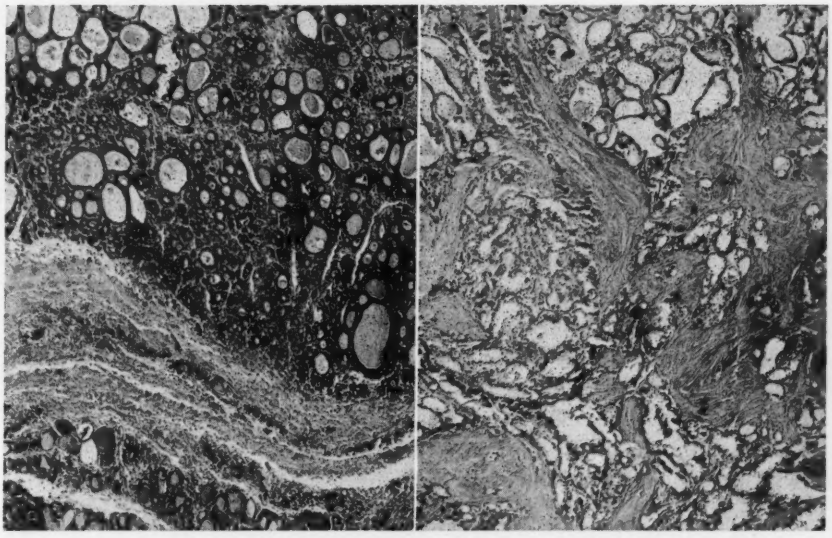

FIG. 1. (left) Typical follicular adenoma, showing intact capsule and numerous immature follicles (×50, H.&E.).

FIG. 2. (right) Follicular carcinoma, showing atypical size and shape of follicles and invasion of stroma (×50, H.&.E.).

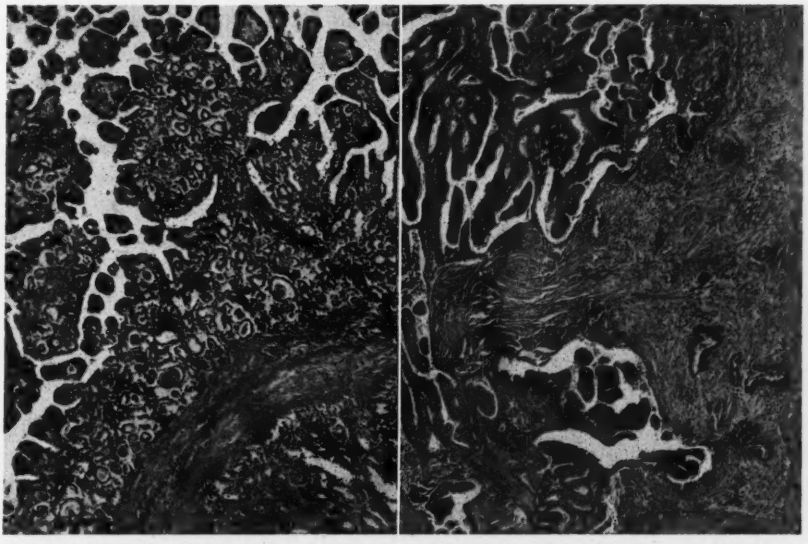

FIG. 3. (left) Papillary adenoma. The papilliferous nature of the tumor is well developed, although a few follicles are formed as well. The capsule in the lower right corner is intact (×50, H.&E.).

FIG. 4. (right) Papillary carcinoma, showing stromal invasion (×50 H.&E.).

tumors were frequent, particularly in the more highly malignant tumors. In such cases of mixed type, the predominant pattern was the one used for classification purposes.

In the cancer tabulation it was found that papillary tumors are by far the most common single type. If one remembers that many cancers not classified as predominantly papillary still have variable amounts of papillae formation, the relative frequency of papillary growth in thyroid cancer is even more striking. As in the benign tumors, analysis of the average age and sex incidence showed little variation between the various groups and subgroups of classifiable tumors. Of note, however, is the fact that in the unclassified group the average age of the patient was higher and the incidence in females slightly lower than in the majority of thyroid cancers. Although an attempt to classify the highly malignant tumors was unsatisfactory there was an impression that some had an embryonal or fetal structure. Mixtures of papillary or follicular structure with undifferentiated growth were sometimes seen.

DISCUSSION

It is a comparison of the tabulation of the benign and malignant tumors which is of most interest. The most striking finding is the great variation in incidence between benign and malignant counterparts. Whereas in benign tumors a follicular structure is common and papillary structure rare (ratio 17:1), the exact reverse is true in the malignant tumors (ratio 1:2). Also of interest is the observation that—anaplastic carcinomas excepted—the age of the patient at the time of removal and the percentage incidence in females are almost identical in all thyroid tumors, regardless of structure and regardless of whether benign or malignant. The ratio with which the subgroups of follicular adenoma are seen in the cancers is about the same as for follicular adenomas when they are considered as a group.

The foregoing material and data are a histologic analysis of surgical pathologic specimens. Tempting as it seems, one must be cautious about drawing definitive conclusions from such statistical studies. There are, however, several comments which seem justifiable.

The great discrepancy between the incidence of benign as opposed to malignant papillary tumors is certainly of some significance. The discrepancy is so great that, at the time of removal, a papillary tumor of the thyroid is many times more likely to be malignant than benign. When one remembers the fact that many follicular thyroid cancers also have papillary foci, the incidence and significance of the papillary pattern of growth in malignant tumors is even greater. The pathologist must view any tumor showing papillae with suspicion and be careful to rule out malignant change before assuming that it is benign. The great discrepancy between the inci-

dence of papillary structure in benign and malignant states suggests that perhaps most papillary carcinomas—the most common form of thyroid cancer—are malignant from their inception and do not arise from a pre-existing benign phase.

From an analysis of the foregoing data there is seen no valid reason for perpetuating the subgroups of tumors of the follicular group, even though they can be readily distinguished. Whether considered separately or as a group, the follicular forms have a comparable age and sex incidence and appear in malignant tumors in about the same relative ratio as in the benign. It would seem that such subdivision serves little useful purpose and well might be discarded in the interest of simplification and clarity.

The regularity and uniformity of the age distribution and sex incidence of thyroid tumors of all types (except the highly malignant) in the data analyzed is remarkable. One gains the impression that the classification of thyroid tumors should be unified rather than broken down into diverse groupings; nor can one resist wondering why, if benign tumors predispose to cancer, the average age of the patient at the time of removal of each is almost identical. The data presented here neither prove nor disprove that thyroid carcinomas arise from pre-existing adenomas but do give rise to the conjecture that many thyroid cancers are, as concluded by Crile (4), malignant from their onset without passing through a benign phase. Such an observation suggests that solitary nodules of the thyroid, as with solitary nodules of the breast, should be removed principally for diagnosis and not only for the prevention of development of cancer.

SUMMARY

After establishing criteria for histologic growth patterns of thyroid tumors, an analysis and comparison of the incidence of such patterns in benign and malignant tumors has been made. Similarities and contrasts not only in the relative incidence of the patterns, but also in the sex and age distribution of the patients are discussed in relationship to the question of the transition of benign to malignant tumors.

REFERENCES

1. Lahey, F. H.; Hare, H. F., and Warren, S.: Carcinoma of the thyroid, *Ann. Surg.* **112**: 977, 1940.
2. Warren, S.: The classification of tumors of the thyroid, *Am. J. Roentgenol.* **46**: 447, 1941.
3. Rawson, R.: *in* Means, J. H., The Thyroid and its Diseases, ed. 2. Philadelphia, J. B. Lippincott Company, 1948.
4. Crile, G., Jr., and Dempsey, W. S.: Indications for removal of nontoxic nodular goiters, *J.A.M.A.* **139**: 1247, 1949.

THE METABOLIC FATE OF I^{131} AFTER LARGE THERAPEUTIC DOSES IN PATIENTS WITH METASTATIC CARCINOMA OF THE THYROID*†

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THE unpleasant fact emerging from the treatment of metastatic carcinoma of the thyroid with I^{131} is that the results are frequently unsatisfactory. Freedberg, Ureles, Lesses and Gargill (1) have reported a single spectacular apparent cure, and Rawson (2) has seen 2 similar cases which were more advanced and in which the results were even more dramatic. We have also seen similar cases but they have been the exceptions. Yet the fact remains that large amounts of I^{131} may be concentrated in certain metastases. Why then are the results unsatisfactory? Certainly a partial answer is that the isotope is not concentrated uniformly in different metastases or even within the same metastasis. Perhaps part of the answer also is that insufficient dosage has been used.

Increasing the dosage of I^{131} beyond certain limits will certainly lead to bone marrow damage. Rawson (2) has reported a single fatality in a patient who received 638 millicuries of I^{131} over a fourteen-month period, from which she received a calculated radiation dosage of 1,300 r.e.p. to the blood. Rawson has suggested a study of the radiation derived from a tracer dose, as an index of what may be expected from a subsequent treatment dose, and has advised that no dose calculated to deliver more than 500 r.e.p. to the blood be given.

The radiation dosage which the patient receives from an administered

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radioactive substance is an involved problem and depends upon the fate of the isotope in the metabolic mill. The complex character of iodide metabolism, especially when further complicated by tissue damage, has led us to an examination of the fate of I^{131} after heavy dosage, in order that the processes may be defined quantitatively and inferences drawn which may bear on matters of dosage technic and safety.

CLINICAL MATERIAL AND METHODS

The subjects of this study were 8 patients with alveolar, or mixed papillary and alveolar carcinoma of the thyroid. All but 2 had had a previous total surgical thyroidectomy: in 1 (Case 5) thyroidectomy was technically impossible. Some had received previous therapeutic doses of I^{131}. Almost all studies were conducted on the metabolic ward of the hospital, and in all, accurate and complete stool and urine collections were possible.

Tracer and therapeutic doses of carrier-free I^{131} were given in water, and the urine collected in bottles kept within a lead safe at the bedside. Measurements of gamma radiation from the patient were made daily, either with an ionization chamber or with a scintillation counter suitably shielded and placed at a standard distance from the patient. Blood was obtained frequently for measurements of radiation, for fractionation into its iodinated components, and for determination of the iodide content. After therapeutic doses the radioiodide content of the serum was measured by placing a sample in a casette in front of a heavily shielded scintillation counter, employing a sodium iodide crystal (3). Aliquots of the serum were separated into the iodinated components by a modification of the tissue method of Taurog, Tong, and Chaikoff (4), in which the successive extractions were measured directly. After the first two days following the therapeutic I^{131}, only a negligible proportion of the I^{131} was present as a nonprecipitable component.

Direct counting of radioactivity in serum was not possible beyond a few days after a large tracer amount of I^{131} (500 microcuries). These samples were digested in chromic acid and then distilled: the distillates were precipitated as silver iodide, collected on filter paper discs, and counted with an end-window counter. Recovery by this method was shown to be better than 90 per cent.

The concentrations of labeled iodine in urine and fecal specimens were determined by measurements of suitable aliquots of the collections in the geometrical center of a four-quadrant scintillation counter (3), and comparison with an appropriate standard.

Starting with the equation which relates dose rate, concentration and average energy of particulate radiation (5),

$$R = 61\ C\overline{E}\ \text{rep/day} \qquad 1$$

where C is in mc./kilogram and $\overline{E}$ is the average energy in MEV, one can readily derive the dosage resulting from the radioiodide phase. Assuming the iodide space is 35 per cent of the body mass (6), and that the thyroid and renal iodide clearances are k_1 and k_2 respectively in percentage of (v_1+v_2) per day:

$$C=\frac{A}{.35\ M}\ e^{-(k_1+k_2)}\ \text{mc./liter} \qquad \text{II}$$

where A is the number of millicuries administered, and M is the mass of the patient in kilograms; v_1 is the plasma volume, and v_2 is a second volume of diffusion of iodide considered to have the same concentration as the plasma, and is therefore a virtual rather than an actual volume. Thus, (v_1+v_2) constitutes a volume of distribution of iodide. It can be found by extrapolation to zero time of the second exponential portion of a semilogarithmic plot of the plasma I^{131}. Clearance, therefore, is expressed as a percentage of this virtual volume cleared of iodide per unit of time.

The constant (k_1+k_2) is given by the relation:

$$k_1+k_2=\frac{0.693}{t_{\frac{1}{2}}} \qquad \text{III}$$

where $t_{\frac{1}{2}}$ is the half-time of the labeled iodide in the plasma in days, and also by the relation:

$$k_1+k_2=\frac{k_2}{E} \qquad \text{IV}$$

where E is the fractional excretion of radioiodide (7). By measurement of $t_{\frac{1}{2}}$ and E, values for k_1 and k_2 can be assigned. For the purpose of the calculations of this paper, k_2 is arbitrarily taken as 7 per cent per hour or 168 per cent per day, and from equation IV:

$$E=\frac{168}{k_1+k_2}\ . \qquad \text{V}$$

By introducing C from equation II into equation I, and integrating from 0 to ∞, the total iodide dosage is found to be:

$$D=21\ \frac{AE}{M}\ \text{rep} \qquad \text{VI}$$

The radiation dosage resulting from the release of labeled hormone was determined by graphic integration of the plasma concentration curves, and the use of the following equation:

$$R=12.4\ CAP\ \text{rep/day} \qquad \text{VII}$$

where C is the fractional concentration of labeled iodine per liter of plasma, A the amount of administered radioiodine in millicuries, and P the physical decay.

The radiation dosage calculated from these equations is that solely from the beta particles. This does not mean that the dosage resulting from gamma rays is entirely negligible. However, the contribution due to gamma rays will depend on the geometric relationships of the part of the body un-

der consideration. An estimate of this contribution would simply add a certain percentage to the beta-ray dosage. Since an arbitrary factor must be included in order to compare the plasma dosage with whole body dosage (such as the generally accepted median lethal dose of approximately 450 roentgens), the use of beta-ray dosages would seem to be most logical. In general, the conversion factor from plasma dosage to whole body dosage appears to be between 2 and 3. It is interesting that the ratio of total body volume to iodide space is also between 2 and 3.

RESULTS

The results of the studies on the 8 patients are shown in Figures 1 through 8, and in Figure 9 are shown the findings with a tracer dose of 500 millicuries of I^{131} immediately preceding the therapeutic dose in Patient 8. Table 1 is a summary of the radiation dosage data.

TABLE 1

Patient No.	Administered dose		Percentage retained at 48 hrs.	I^{131} (r.e.p.)	PBI^{131}* (r.e.p.)	Max. blood level (% adm. dose/liter serum)
	(mc. I^{131})	(mc./Kg.)				
1	100	2.54	80.1	10.7	70.0	2.12
2	200	2.34	58.8	20.2	212.4	2.49
3	200	2.45	11.1	45.0	31.1	.24
4	200	3.20	20.0	54	42.1	.33
5	200	2.24	40.1	56.6	33.4	.43
6	250	2.78	44.3	32.4	70.2	2.47
7	200	3.20	85.7	10.1	89.2	.85
8	300	3.81	3.0	90.5	12.8	.97

* First nine days.

Case 1 (*MGH #758213*). A 24-year-old female had had a hemithyroidectomy seven years previously for a mixed papillary adenocarcinoma; examination showed residual firm thyroid tissue in the neck. Her weight was 39.2 kilograms. A roentgenogram of the chest showed numerous round homogeneous nodules throughout both lung fields. A tracer of I^{131} disclosed patchy localization of activity in the neck and over the lung fields. The concentration of serum protein-bound iodine on entry was 5.6 micrograms per 100 cc. A total thyroidectomy was performed. Shortly thereafter the patient received a dose of 100 millicuries of I^{131}, 19.9 per cent of which was excreted in the first forty-eight hours. During subsequent months there was remarkable, but incomplete, clearing of the pulmonary metastases. Four months after operation her retention of a tracer of I^{131} was 63.1 per cent. The findings in this case are illustrated in Figure 1.

After an initial drop in the excretion rate of I^{131} to 0.82 per cent of the administered dose per day, there was a rise to a maximum of 4.1 per cent per day on the ninth day.

There was a concomitant rise in the total radioactivity of the serum, from a minimum of 0.32 per cent of the administered dose per liter of serum at the end of the first day, to a maximum of 2.12 per cent per liter on the sixth day. The calculated radiation received by the blood during the iodide phase was 10.7 r.e.p. and that received during the PBI^{131} phase for the nine days of observation was 70.0 r.e.p. During this time there was a drop in the total white blood cell count from 7,675 to 6,200 (a fall of 19.2 per cent) and in the lymphocyte count from 2,226 to 868 (a fall of 61 per cent).

Case 2 (*MGH #683420*). A 59-year-old woman had undergone a total thyroidectomy two years previously, at which time a diagnosis of carcinoma of the thyroid with pelvic

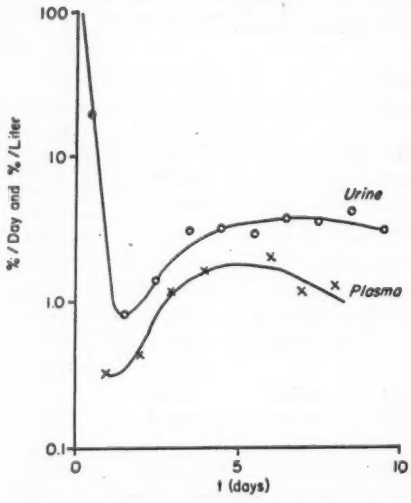

FIG. 1. Case 1. Appearance of I^{131} in the serum (per cent per liter) and in the urine (per cent per day) after the administration of 100 millicuries to a patient with carcinoma of the thyroid.

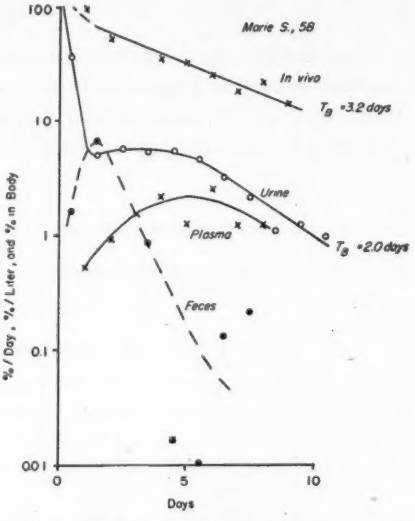

FIG. 2. Case 2. Fate of a therapeutic dose of 200 millicuries of I^{131} in a 59-year-old woman with metastatic carcinoma of the thyroid. The *in vivo* figures represent the percentage of the administered dose present in the whole body at forty-eight hours. The fecal excretions are expressed as the percentage of the administered dose excreted on a specific day.

metastases had been made. The histologic diagnosis, based on findings in a biopsy specimen removed from the pelvis, was alveolar carcinoma. She had received several courses of therapeutic radioiodide; the last dose had been 127 millicuries administered five months previously. The serum protein-bound iodide level was 4.5 micrograms per cent, and 19.2 per cent of a tracer dose of radioactive iodide was excreted in the first forty-eight hours. Accordingly, the patient, who weighed 85.2 kilograms, received a therapeutic dose of 200 millicuries of I^{131}. Of this, she excreted 41.2 per cent in the first forty-eight hours.

There was no secondary rise in the urinary excretion rate (Fig. 2). After excreting at

approximately the same rate for five days, there began a decline in the rate, which approached a half-period of 2.0 days. At the same time there was a sharp rise in the blood level of radioactivity, to a maximum of 2.49 per cent of the administered dose per liter of serum on the sixth day, following which there was a decline. There was a high fecal excretion during the first four days, the maximum level being 6.8 per cent of the administered dose on the second day. The decline in the labeled iodine in the patient as a whole, had a half-period of 3.2 days. The radiation received by the blood during the iodide phase was 20.2 r.e.p., whereas that received during the PBI^{131} phase in the succeeding nine days was 212.4 r.e.p., for a total dose during this period of 232.6 r.e.p. There was a fall in total white blood cell count from 4,500 to 3,150 and in lymphocyte count from 1,461 to 315—decreases of 30 per cent and 78.4 per cent, respectively. Unfortunately, neither this patient nor the preceding one was followed long enough to see what the ultimate slopes of the various curves might be. It is surprising to note the high fecal excretion in this patient, since no other patient in this group excreted more than 1.5 per cent of the administered dose during any single day.

Case 3 (*MGH #758220*). A 49-year-old male noticed a mass on the upper sternum about six months before admission. His physician discovered a nodule in the thyroid which proved to be adenocarcinoma; subsequently a total thyroidectomy was performed. On admission there was a 6-centimeter dome-shaped mass over the upper sternum. Punch biopsy showed adenocarcinoma of the thyroid. The patient retained 51.5 per cent of a tracer dose of I^{131}. After an unsuccessful trial at stimulation of the tumor with thyrotropic hormone, the patient received 200 millicuries of I^{131}, of which he retained only 11 per cent. The serum protein-bound iodine level was 0.8 microgram per cent.

After the first few days the disappearance of the radioactive iodide from the urine pursued a regular course, with a half-period of 4.7 days, which was nearly the same as the rate of disappearance of the labeled iodide from the body as a whole, which had a half-period of 5.0 days (Fig. 3). After the initial fall in the blood level during the iodide phase, there was a secondary rise to a peak of 0.24 per cent of the administered dose per liter of serum on the fourth day. After this there was a fall and, finally, a leveling off at almost no change from day to day. The fecal excretion pursued a course which was parallel with that of the urine and whole body. During the iodide phase the blood received 45.0 r.e.p. of beta radiation and during the first nine days of the PBI^{131} phase, a total of only 31.1 r.e.p.

The surprising finding here and in most of the other patients was the lack of parallelism between the blood curves on the one hand and the whole-body and urine curves on the other. In this patient most of the radiation was delivered to the blood within the first twenty-four hours and subsequent radiation to the blood was negligible, whereas the reverse occurred in the previous 2 patients. There was a drop in the white blood cell count from 7,750 to 4,125, and in the lymphocyte count from 2,625 to 1,500—decreases of 46.7 per cent and 42.8 per cent, respectively.

Case 4 (*MGH #757026*). A 55-year-old married female had had an "adenoma" of the thyroid removed six years previously. She was admitted to the hospital because of pain in the right hip arising from a metastatic lesion which was destroying the right femoral neck and head. A punch biopsy of this lesion revealed metastatic adenocarcinoma from the thyroid. A total thyroidectomy was performed and after an unsuccessful attempt at enhancing radioactive iodide uptake with thyrotropic hormone, the patient received 200 millicuries of I^{131}. The immediately preceding tracer had shown an excretion of 83.2 per cent in the first forty-eight hours.

The urinary excretion of the I^{131} was not a simple process (Fig. 4). The total excretion

in the urine was somewhat in excess of 100 per cent, apparently as a result of error in the initial collection. During the first three days the I^{131} was excreted rapidly, but this gave way to a much lower excretion which had a biologic half-period of 4.2 days, a slope approximately the same as that for the body as a whole (3.9 days). There was an initial rapid fall in the blood level, followed by a slow, regular fall with a half-period of 12.6 days. The fecal excretion was extremely low.

Here again the lack of parallelism between the blood curve and the urine and whole-body curves is apparent. During the iodide phase a radiation dose of 54 r.e.p. was delivered to the blood and during the PBI^{131} phase, 42.1 r.e.p. There was a fall in the white

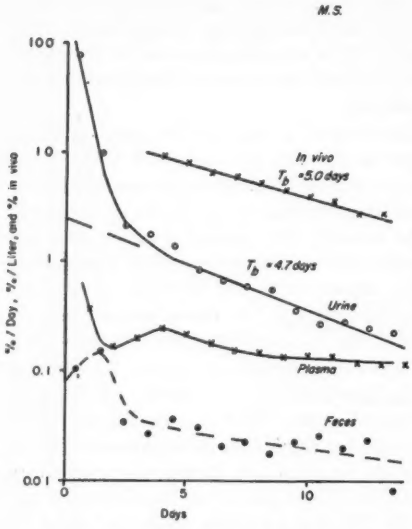

FIG. 3. Case 3. Fate of a 200-millicurie dose of I^{131} in a 49-year-old male with a sternal metastasis from carcinoma of the thyroid. (Charting as in Figs. 1 and 2.)

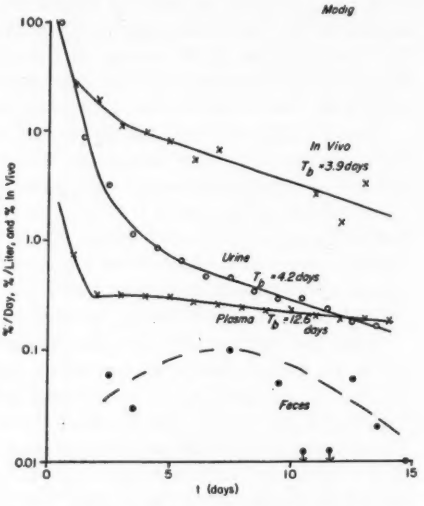

FIG. 4. Case 4. Slight retention of a 200-millicurie therapeutic dose of I^{131} in a 55-year-old woman with carcinoma of the thyroid metastatic to the hip. (Charting as in Figs. 1 and 2.)

blood cell count from 8,750 to 5,775, and in lymphocyte count from 2,406 to 1,024—decreases of 34 per cent and 57.4 per cent, respectively.

Case 5 (*MGH #744775*). A 14-year-old female had had an enlargement of her cervical lymph nodes for at least three years. Finally a biopsy of one of these disclosed the presence of a mixed papillary and alveolar carcinoma of the thyroid, which was metastatic to the cervical nodes. X-ray study of the lungs revealed extensive metastatic areas throughout both lung fields. An attempt at surgical removal of the remaining normal thyroid was impossible because of the infiltration of vital structures. She excreted 59.8 per cent of a tracer dose of I^{131}, and there was considerable retention of the isotope over the lung fields as well as in the neck. She was given a 200-millicurie dose of I^{131}.

After an initial fall in the excretion rate, there was a rise to a maximum of 3.2 per cent of the administered dose per day on the fourth day (Fig. 5). Subsequently, the excretion rate fell and assumed a constant rate with a half-period of 3.0 days, which paralleled the

rate of disappearance of labeled iodide from the body as a whole, which had a half-period of 3.0 days. The blood level fell rapidly, then rose to a maximum concentration of 0.45 per cent of the administered dose per liter, and then assumed a constant rate of fall with a half-period of 8.5 days. The fecal excretion was irregular because of constipation.

This patient retained 40 per cent of the administered dose for the first forty-eight hours; nevertheless, the radiation dose delivered to the blood during the iodide phase was 56.6 r.e.p. and during the first nine days of the PBI^{131} phase, only 33.4 r.e.p. The total white blood cell count fell from 12,800 to 5,200, and the lymphocyte count from 3,456 to 693—decreases of 59.3 per cent and 79.9 per cent respectively.

Case 6 (*MGH #624607*). This 43-year-old married woman had first been admitted two years previously with a diagnosis of adenocarcinoma of the thyroid metastatic to the

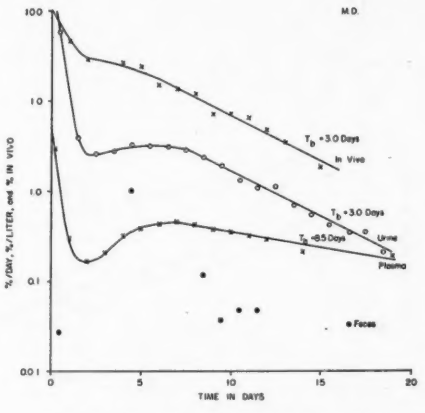

FIG. 5. Case 5. Metabolic fate of 200 millicuries of I^{131} in a 14-year-old girl with widespread metastatic thyroid carcinoma. (Charting as in Figs. 1 and 2.)

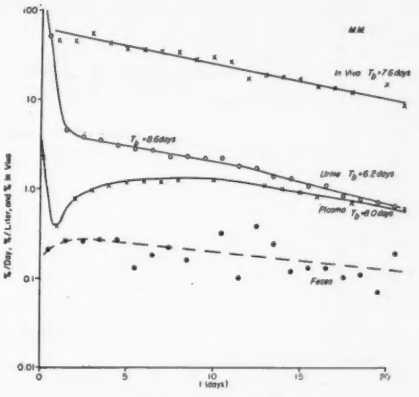

FIG. 6. Case 6. Metabolic fate, in a 43-year-old female, of 250 millicuries of I^{131} administered for treatment of metastatic thyroid carcinoma. (Charting as in Figs. 1 and 2.)

ribs, lumbar vertebrae, pelvis and left humerus. A total thyroidectomy had been performed and subsequently she underwent many studies and many therapeutic trials with varying doses of radioactive iodide. Following a trial of constant intravenous thyrotropic hormone, which elevated her 48-hour I^{131} retention from 51.2 per cent to 71.0 per cent, she received a therapeutic dose of 250 millicuries of I^{131}.

After the initial fall in excretion of iodide, the daily excretion rate assumed a steady fall with a half-period of 8.6 days for the first twelve days, following which it assumed a new and slightly more rapid slope, with a half-period of 6.2 days (Fig. 6). These values were essentially parallel with the rate of disappearance from the body as a whole, which had a half-period of 7.6 days. The blood curve, however, rose rapidly after the first day to reach a maximum of 1.25 per cent of the administered dose per liter of serum on the tenth day, after which it slowly fell to assume a steady rate of decline with a half-period of 8.0 days. Fecal excretion was quite variable, as might be expected. The radiation dosage delivered to the blood during the iodide phase was 32.4 r.e.p., and during the first nine days of the PBI^{131} phase, 70.2 r.e.p. There was a change in white blood cell

count from 8,025 to 6,850, and a fall in lymphocyte count from 1,605 to 664 (58.6 per cent).

This patient, with a relatively high retention of I^{131}, experienced a very prolonged rise in the blood radioiodide level. After approximately two weeks the decline of labeled iodine from the body as a whole paralleled the fall in the blood and in the excretory rate in the urine.

Case 7 (MGH #80959). An 83-year-old woman had had an excision of a mass in the

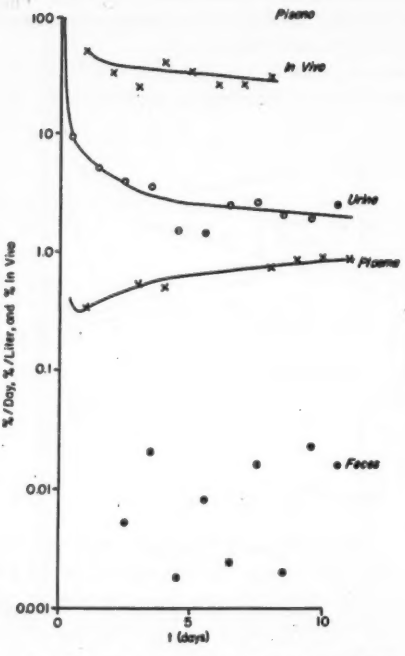

FIG. 7. Case 7. Metabolic fate of 200 millicuries of I^{131} in an 83-year-old woman, with widespread metastatic carcinoma of the thyroid. Excretion by the fecal route was extremely low. (Charting as in Figs. 1 and 2.)

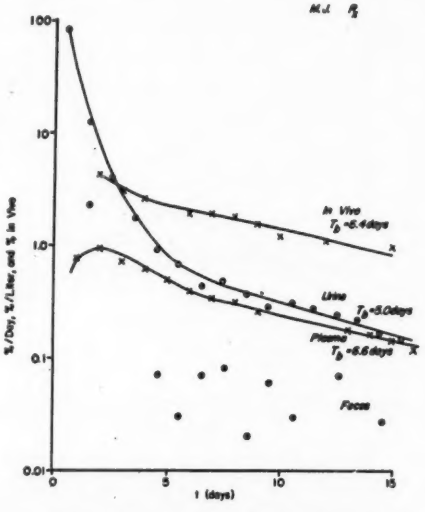

FIG. 8. Case 8. Metabolic disposal of 300 millicuries of I^{131} in a 60-year-old woman with carcinoma of the thyroid metastatic to the hip. The low retention is surprising in view of the preceding trace dose (Fig. 9). (Charting as in Figs 1 and 2.)

right neck two years previously, which was reported to show alveolar carcinoma of the thyroid. For several months she had been aware of a tumor of the left mandible, a biopsy of which also revealed alveolar cell carcinoma. X-ray studies disclosed extensive metastatic destruction of the left ilium and sacrum, as well as the mandible. Several metastases were seen in the ribs. The initial operation consisted of a left total and a right subtotal thyroidectomy. The serum protein-bound iodine level was 6.4 micrograms per cent. Study with a tracer of I^{131} showed localization in the metastatic areas, and accordingly she was given 200 millicuries of radioactive iodine. She excreted only 15 per cent of this during the first forty-eight hours.

There was a slow release of labeled iodine in the urine and a comparable slow fall in the total body content (Fig. 7). Concomitantly the I^{131} content of the plasma rose slowly throughout the entire period of observation. The blood received a total radiation dose of 10.1 r.e.p. during the I^{131} phase, and 89.2 r.e.p. during the first nine days of the PBI^{131} phase. The white blood cell count changed from 6,600 to 5,100, while the lymphocyte count fell from 1,101 to 589 (46.5 per cent). Unfortunately, this patient was not followed for a long enough period for an accurate assessment of the half-periods, either from the total body count or from the urinary excretion. Inspection of the curves, however, suggests that these rates were approximately parallel.

Case 8 (*MGH#752641*). A 60-year-old married woman was admitted to the hospital because of pain in the left thigh during the previous year. A mass was found in the left lobe of the thyroid, and by x-ray examination a large, soft, tissue mass was seen to pro-

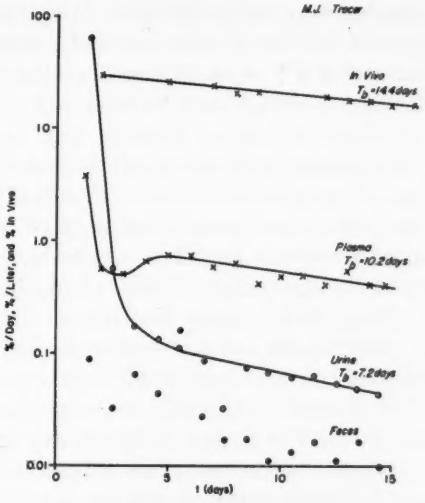

FIG. 9. Case 8. Fate of a tracer of 500 microcuries of I^{131} given to the subject of Figure 8. (Charting as in Figs. 1 and 2.)

trude into the pelvis in the region of the left acetabulum. The specimen obtained at the time of total thyroidectomy showed adenocarcinoma of the thyroid. Tracer study with I^{131} showed localization of a moderate degree of activity over the left hip. She excreted 81 per cent of a 500-microcurie tracer dose; and two weeks later, nearly all of a 300-millicurie therapeutic dose. There was no evident reason for the change in retention, other than possible effects from the 500-microcurie tracer dose.

There was a rapid elimination of labeled iodine in the urine during the first forty-eight hours, but the change in rate slowly approached a half-period of 5.0 days (Fig. 8). The total body content had a half-period of 6.4 days; and the plasma, after an initial secondary rise, eventually had a half-period of 6.6 days. The fecal excretion rate was very low. The measurements with the preeeding tracer dose gave a half-period of 14.4 days for the total body decay rate, 10.2 days for the disappearance from the blood, and 7.2 days for

the fall in urinary excretion rate. During the I^{131} phase of the therapeutic dose, a radiation dose of 90.5 r.e.p. was delivered to the blood, and during the first nine days of the PBI^{131} phase, 12.8 r.e.p. There was a change in white blood cell count from 6,175 to 5,350, and a fall in the lymphocyte count from 1,853 to 1,177 (36.5 per cent).

DISCUSSION

From a study of these patients a consistent pattern seems to emerge. After an initial period of adjustment occupying the first two or three days, the curve of labeled iodine retained by the patient assumed a simple exponential form, *i.e.*, a straight line on semilogarithmic paper. The rate of fall was surprisingly high. Customarily, after diagnostic or therapeutic amounts of I^{131} for thyrotoxicosis, biologic half-periods of fifteen to one hundred days are encountered. For the rapid rates found in this study, which varied from three to twelve days, several explanations can be offered. Re-utilization of the I^{131} released from breakdown of the protein-bound labeled iodine of the plasma may be inhibited by the initial impact of the therapeutic dose on the tumor. Another explanation is that the released PBI^{131} may be broken down so slowly in the periphery that it is not available for re-utilization by the tumor. The radiation delivered to the tumor might accelerate the release rate of fixed I^{131}. If this is true, it is difficult to see why the half-period should be short both in subjects with little evidence of biologic effect on the tumor, and in subjects with tumor damage, as indicated by x-ray evidence of diminution of metastases (Case 1) or by high blood levels of protein-bound I^{131}. We have also observed short half-periods after tracer doses in carcinoma of the thyroid. This suggests that rapid turnover is a property of the tumor.

Urinary excretion of I^{131}: The largest daily urinary excretion of the administered dose of labeled iodine occurred within the first twenty-four hours, and was much less during the second twenty-four hours. After this there was either a sharp leveling-off of the excretion rate to a rate of decline comparable to that for the body as a whole, or there was a secondary rise which extended through the fifth or sixth day followed by a decline with a half-period comparable to that of the body as a whole. The secondary rise did not occur necessarily in the patients with the most striking secondary rises in blood content.

Blood I^{131} levels: The curves of concentration of I^{131} in the blood proved interesting and unexpected. In general, the blood concentration fell rapidly during the first twenty-four hours, as the radioiodide was cleared from the blood by the kidneys and the metastases. The concentration then rose, reaching a peak at two to ten days after administration of the dose. Thereafter it fell on a fairly smooth curve. These findings are similar to those in a patient studied by Seidlin (9). Qualitatively, this was what would be

expected on the basis of the usually assumed models of thyroid function. The secondary rise in the blood level results from the iodide which enters the thyroid, is bound organically and is gradually released, building up the blood concentration of protein-bound I^{131}. The gradual fall results as the thyroid store of labeled iodine is depleted, with a consequent decrease in the store in the serum. In comparing the fate of therapeutic amounts of radioiodine in metastases with the fate of tracer amounts of radioiodine in the normal thyroid, it is not the similarities but the differences that are of interest. It would be highly presumptive to assume that the untreated thyroid metastasis has an iodine metabolism identical with that of the normal thyroid. This does not mean, however, that the study of the fate of therapeutic doses of I^{131} in these patients will not yield useful information about the processes involved. Aberration from the normal iodine picture, or even the variations among patients when correlated with clinical response, may well provide data on the progress and success of the treatment.

The striking finding in our study was the failure of the blood curves to follow the urinary and the whole-body curves from the second to the fifteenth days. The half-periods of the blood curves were regularly longer. The data shown in Figure 6, obtained over a longer period of time, show the blood curve gradually assuming a new slope more nearly that of the urinary excretion and whole-body retention curves. There are several possible explanations for the deviations among these curves.

1. There may be renal damage during the iodide phase of the blood concentration curve, *i.e.*, the first twenty-four hours. Although the urinary excretion curve is plotted in terms of percentage per day, if it is assumed that the daily urinary volume is constant, this curve is proportional to the radiation dosage which the kidney tubules receive, neglecting the factor of radioactive decay.
2. Radiation damage to the metastatic lesion may produce increased permeability of the thyroid parenchyma for protein-bound I^{131}, or in some other way lead to an increased secretion rate of labeled hormone.
3. The metastasis, as a result of radiation damage, may liberate labeled compounds which are metabolized in a manner differing from that of the normal thyroid hormone.

There may be other explanations for the observed phenomenon, but in all probability the foregoing second and third factors are operative. We have not yet detected any evidence of kidney damage. Recently, Tong, Taurog and Chaikoff (8) and Rawson (2) have reported the presence of non-butanol-extractable substances in the blood of patients treated with therapeutic doses of I^{131}. By similar extraction procedures we have been able

in these patients to confirm the presence of an abnormal I^{131}-containing substance in the blood after heavy therapeutic doses.

Fecal excretion of I^{131}: The fecal excretion rates were highly irregular. One patient excreted 6.8 per cent of the administered dose in a single day, but for the most part the excretions by this route were less than 0.1 per cent per day, and the total for the period of observation was less than 1 per cent.

Dosage considerations: The radiation delivered to the blood is derived from two phases. The first of these, the iodide phase, is from the I^{131} before it has been taken up by the tumor. The second is from protein-bound I^{131} released by the tumor. The first phase has practically ended by the second day. The blood radiation during this phase depends on the rate of removal of I^{131} by the tumor, the rate of excretion by the kidney, and on the volume of distribution of the iodide, which is approximately 35 per cent of the body weight (6). In the patients in this study the blood radiation dose during the iodide phase varied inversely with the 48-hour retention, because in patients with high retention the I^{131} was being removed rapidly from the blood.

The second phase of blood radiation is much more protracted because of the constant replenishment of the blood by the PBI^{131} stored in the tumor and because of the slow rate of breakdown of the I^{131}-containing precipitable substances of the blood. The blood radiation from this phase is complex and evidently dependent upon a variety of factors, including absolute dose, tumor mass, and the radiation sensitivity of the tumor.

There were no consistent relationships between the amount of blood radiation received from the PBI^{131} phase, the initial administered quantity of I^{131}, or the initial 48-hour retention. Two patients who had high retentions and who received large therapeutic doses (200 millicuries each) received relatively small amounts of blood radiation, whereas others with equivalent retentions and therapeutic doses received large amounts of blood radiation. It was apparent, however, that the blood radiation received during the I^{131} phase was small, and in these patients it was not over 30.1 r.e.p. per 100 millicuries of administered I^{131}. In some of the patients much heavier radiation doses were received during the PBI^{131} phase. Thus, in Case 2 the patient received only 20.2 r.e.p. during the I^{131} phase but 212.4 r.e.p. during the PBI^{131} phase. Unfortunately, it seems difficult to predict the magnitude of the blood dose. Although doubling the administered quantity of I^{131} would double the radiation dose during the I^{131} phase, it might more than double the dose during the PBI^{131} phase because of increased tumor necrosis.

Radiation effects: In 3 of the patients a mild degree of nausea and vomiting developed. In all 3 it came on about six hours after ingestion of I^{131},

and in 1 it was prolonged more than a few hours. The amount of I^{131} lost in the vomitus was negligible, except in Case 5, in which instance 1.5 per cent of the administered dose was lost.

In all patients there was a fall in the number of total white blood cells, and especially in the number of lymphocytes. In none was there an alarming depression of cells, and in all a rise began in about a week. Characteristic pyknosis of the lymphocytic nuclei was regularly encountered. An effort was made to correlate the white cell changes with the amount of the initial doses of I^{131}, with the blood radiation doses from the I^{131} phase and from the PBI^{131} phase, with the maximum PBI^{131} blood level, and with the total radiation dose. Correlation was poor in all, but the closest relationship was between the fall in the lymphocyte count and the total blood radiation dose.

CONCLUSIONS

1. The metabolic fate of large therapeutic doses of I^{131} in 8 patients with metastatic carcinoma of the thyroid is described.

2. After initial periods of adjustment the radiation measured from the the body as a whole followed a simple exponential decay, with biologic half-periods which varied from three to twelve days.

3. There was a rapid fall in the urinary excretion rate of I^{131} for the first day or two, and in some patients this was followed by a secondary rise. In all those followed for a sufficiently long period, the slope of this curve approached that of the rate of disappearance of I^{131} in the body as a whole.

4. The rapid fall of the iodide phase of the blood curve was superseded by a secondary rise and an adjustment to a simple exponential decay rate, which had a biologic half-period appreciably longer than that of the urine.

5. The significant radiation dosage to the blood may be delivered during the protein-bound I^{131} phase of the blood curve, and is dependent upon a number of factors besides the quantity of the isotope administered to the patient and the retention during the first forty-eight hours.

REFERENCES

1. Freedberg, A. S.; Ureles, A. L.; Lesses, M. F., and Gargill, S. L.: Pulmonary metastatic lesion successfully treated with radioactive iodine, *J.A.M.A.* **144**: 16–19, 1950.
2. Rawson, R. W.; Rall, J. E., and Peacock, W.: Limitations and indications in the treatment of cancer of the thyroid with radioactive iodine, *J. Clin. Endocrinol.* **11**: 1128–1142, 1951.
3. Brownell, G. L., and Stanbury, J. B.: Instrumentation for thyroid measurements, *J. Clin. Endocrinol. & Metab.* (in press)
4. Taurog, A.; Tong, W., and Chaikoff, I. L.: The monoiodotyrosine content of the thyroid gland, *J. Biol. Chem.* **184**: 83–97, 1950.

5. Evans, R. D.: Tissue dosage in radioisotope therapy, *Am. J. Roentgenol.* **58:** 754–756, 1947.
6. Keating, F. R., Jr., and Albert, A.: The metabolism of iodine in man as disclosed with the use of radioiodine, *Recent Progr. Hormone Research* **4:** 429–481, 1949.
7. Brownell, Gordon L.: Analysis of techniques for the determination of thyroid function with radioiodine, *J. Clin. Endocrinol.* **11:** 1095–1105, 1951.
8. Tong, W.; Taurog, A., and Chaikoff, I. L.: Nature of plasma iodine following destruction of the rat thyroid with I^{131}, *J. Biol. Chem.* **195:** 407–413, 1952.
9. Seidlin, S. M.: Radioiodine in the treatment of metastatic thyroid carcinoma, *Med. Clin. North America* **36:** 663–680, 1952.

THE EFFECT OF LOW IODINE DIET ON THYROID FUNCTION IN THE RAT*†

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IT HAS long been known that normal thyroid function is dependent upon an adequate intake of dietary iodine. In more recent years many studies have been made in an attempt to elucidate the exact role iodine plays in the development and function of the thyroid gland. The vast amount of data obtained on this subject has been amply discussed in many textbooks and review articles and will not be considered in detail in this report.

In rats, as in most other vertebrates, thyroid changes develop when the dietary level of iodine is low. A deficiency in iodine intake produces a decrease in the synthesis of thyroid hormone and results in the formation of an hyperplastic goiter. The administration of iodine under such circumstances corrects this deficiency, and the gland assumes a more normal structure. In 1933 Levine, Remington and Von Kolnitz (1, 2) developed a low iodine diet which produced goiter in rats within five weeks. By the use of this diet and with replacement of iodine with potassium iodide, they concluded that the smallest amount of iodine necessary to prevent significant thyroid enlargement was from 1 to 2 micrograms per rat per day.

Remington and Remington (3) found that 265 micrograms of iodine per kilogram in an otherwise well balanced diet will protect growing rats from iodine deficiency. Moreover, growth of the animals was not affected by amounts of iodine fifty to one hundred times what they considered the protective dose.

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‡ Special Research Fellow of the National Cancer Institute of the National Institutes of Health.

Following the work of the foregoing investigators, others have attempted to elucidate the role of iodine in thyroid function, using various types of diets. Many of these diets contained large amounts of soybean meal, which contains a goitrogen. In view of our present knowledge of positive goitrogens, the results obtained in these studies are difficult to evaluate.

Although many theories have been advanced as to how iodine suppresses thyroid enlargement, the exact mechanism remains unknown. The most generally accepted concept is that iodine partially inhibits the production of the thyroid-stimulating hormone or its action upon the thyroid gland. This theory is supported by the work of many investigators (Astwood (4), Rawson and Money (5)).

The following report was undertaken to determine with modern techniques the effect of a low iodine diet on the growth and function of the thyroid gland. No known goitrogen was present in this diet, and therefore changes observed in thyroid function are probably either direct or indirect effects of a low iodine intake, However, the possibility exists that some other factor present or absent in the diet may influence thyroid function.

MATERIALS AND METHODS

About 300 healthy adult male rats of fairly uniform weight of the Sprague-Dawley strain were used in this study. The low iodine diet used was essentially that of Remington and Remington (3). Its composition was as follows: Quaker yellow corn meal (76 parts), wheat gluten flour (Battle Creek Food Company) (20 parts), Fleischmann's irradiated yeast (0.2 part), sodium chloride C. P. (1 part), and calcium carbonate C. P. (3 parts). Unless otherwise noted, ordinary drinking water was supplied freely to all animals. Control animals were given Purina laboratory chow.

Twenty-four hours before autopsy all rats were given 1.0 microcurie of carrier-free I^{131} intraperitoneally. At autopsy animals were anesthetized with pentobarbital and exsanguinated from the dorsal aorta. Thyroids were removed, dissected free of fat and connective tissue and weighed on a Roller-Smith balance. They were then placed in 10-cc. vials containing 5 cc. of Bouin's solution. Counting of I^{131} radiation was performed with a Texas Company gamma-ray Geiger-Müller tube. The I^{131} results have been expressed as the percentage of the administered dose of I^{131} (compared with a standard) which localized in the thyroid.

The serum protein-bound iodine content was determined by the method of Barker (6) as modified by Nesh and Peacock (7). Total thyroid iodine content was determined using essentially the same technique.

EXPERIMENTAL RESULTS

Five different experiments were carried out in this study. In the first four experiments we used the change in thyroid weight and the collection

of radioactive iodine by the thyroid as an indication of change in thyroid status. Thyroidal collection of I^{131} was considered a very sensitive index of thyroid functional change and, along with thyroid weight, gave an indication of possible positive goitrogens which might be present in our diet. The fifth experiment was a more detailed study, showing the changes produced in thyroid growth and function over an extended period of time. The data for these studies are shown in the tables and figures.

Experiment I: The influence of short-term treatment with low iodine diet on thyroidal collection of I^{131}

Four groups of rats (10 animals per group) were used, as shown in Table 1. Group 1 was given regular laboratory chow (Purina) and tap water. Group 2 was given tap water and the low iodine diet. Group 3 received

TABLE 1. EFFECT OF DIETARY IODINE (TEN DAYS) ON THYROID GLAND OF RAT

Diet	Body wt. (Gm.)	Thyroid wt. (mg.)	$\frac{\%\ I^{131}}{\text{Thyroid}}$
Purina diet; tap water	428	22.2	11.4
Low iodine diet; tap water	386	23.8	42.3†
Low iodine diet; distilled water	374	22.9	37.6†
Low iodine diet; double-distilled water	373	25.1*	40.6†

* Indicates p value of <0.05.

† Indicates p value of <0.01 calculated according to method of Fisher (15). Such values are given only when individual determinations were made and samples were not pooled before being analyzed.

regular distilled water and the low iodine diet. Group 4 was given double-distilled water (distilled with NaOH to remove iodine) and the low iodine diet. The iodine-deficient diet was given for ten days, then the I^{131} was injected and autopsy performed twenty-four hours later.

As may be seen from the data, neither of the diets produced any marked change in thyroid weight. Thyroidal I^{131} collection in the controls was 11.4 per cent, whereas it was 42.3 per cent in the animals receiving the low iodine diet and tap water. Distillation of the water had no marked influence on radioiodine collection. Therefore, in all subsequent experiments city tap water was used.

Experiment II: The influence of omitting various dietary components on thyroidal collection of I^{131}

The results recorded in the preceding section suggested the possibility that some component of our diet might be enhancing the collection of radio-

iodine by the thyroid. We, therefore, set up a study maintaining groups of rats (10 animals per group) on diets from which one of the original dietary components had been omitted. The only factor common to all diets was the corn meal. The result of maintaining groups of animals on these various diets for ten days is shown in Table 2. It will be noted that the omission of any portion of the diet decreased the collection of I^{131} by the

TABLE 2. EFFECT ON THYROID OF OMITTING VARIOUS COMPONENTS OF LOW IODINE DIET

Diet	Body wt. (Gm.)	Thyroid wt. (mg.)	$\frac{\%\ I^{131}}{\text{Thyroid}}$
Low iodine diet	339	23.5	69.2
No NaCl	344	22.2	54.8*
No wheat	365	24.9	48.7*
No yeast	349	23.6	48.1*
No $CaCO_3$	368	21.8	38.0*

* *See* footnotes to Table 1.

thyroid, although the gland weight was not significantly changed. The most striking change in I^{131} collection was found when calcium carbonate was omitted from the diet. These results strongly suggested that the low iodine diet we were using did not contain any known goitrogens.

Experiment III: The time response of the thyroid to the diet

Five groups (6 rats per group) were set up to investigate the change induced in thyroidal I^{131} collection after animals had been on a specific diet for various periods of time. A sixth group of animals received the control

TABLE 3. THE TIME-RESPONSE OF THE THYROID TO THE DIET

Days on diet	Body wt. (Gm.)	Thyroid wt. (mg.)	$\frac{\%\ I^{131}}{\text{Thyroid}}$
2	395	15.7	17.2†
4	369	18.0	19.6†
6	376	16.3	26.7†
8	369	16.3	30.7†
10	373	17.3	31.0†
Controls	*411*	*18.6*	*10.0*

† *See* footnotes to Table 1.

diet. The times chosen for the duration of the low iodine diet treatment were two, four, six, eight and ten days. As Table 3 shows, no significant change occurred in the weight of the thyroid gland. The collection of I^{131} was, however, markedly changed within forty-eight hours after the diet was started. The data suggest a definite time response for the amount of I^{131} picked up by the thyroid.

Experiment IV: The effect of daily administration of iodide on animals receiving a low iodine diet

Groups of animals (10 rats per group) were maintained for ten or twenty days on the low iodine diet. Various groups of rats received daily doses of 1, 5, 10, or 50 micrograms of iodine as potassium iodide by intraperitoneal injection. The results obtained in this experiment are shown in Table 4 and Figure 1. It can be seen that ten days of treatment with the low iodine diet did not significantly change thyroid weight, but treatment with in-

TABLE 4. THE EFFECT OF DAILY ADMINISTRATION OF IODINE TO ANIMALS ON LOW DIETARY IODINE

Amt. of iodide administered	Days on diet	Body wt. (Gm.)	Thyroid wt. (mg.)	$\frac{\%\ I^{131}}{\text{Thyroid}}$
Purina diet group	—	249	13.2	13.6
Control diet group	10	231	14.0	28.9
1 gamma/day	10	217	12.7	29.1
5 gamma/day	10	220	12.4	16.5
10 gamma/day	10	220	11.9*	10.8†
50 gamma/day	10	224	11.4	3.2†
Purina diet group	—	266	13.7	5.7
Control diet group	20	232	14.1	26.7
1 gamma/day	20	223	12.2	25.4
5 gamma/day	20	229	13.8	12.9†
10 gamma/day	20	226	10.9†	7.4†
50 gamma/day	20	223	10.8*	2.6†

* and † *See* footnotes to Table 1.

creasing amounts of iodine (as KI) gave results which suggest that this regimen caused some decrease in the size of the gland. However, twenty days of treatment with the low iodine diet and various doses of iodide produced only a slight decrease in thyroid weight beyond that found at ten days. I^{131} collection by the thyroids of control animals (Purina diet)

at ten days was 13.6 per cent of the administered dose. In the animals on low iodine intake, the collection had increased to 28.9 per cent, and injection of 1 microgram of iodine daily did not change this uptake. Higher doses of KI produced a progressive decrease in the collection of I^{131}. At twenty days the group receiving the Purina diet showed a thyroidal collection of about 6 per cent of the administered I^{131}. Although this amount of I^{131} was much lower than that usually collected in animals otherwise untreated, it is not a rare finding. The normal thyroidal uptake of I^{131} is

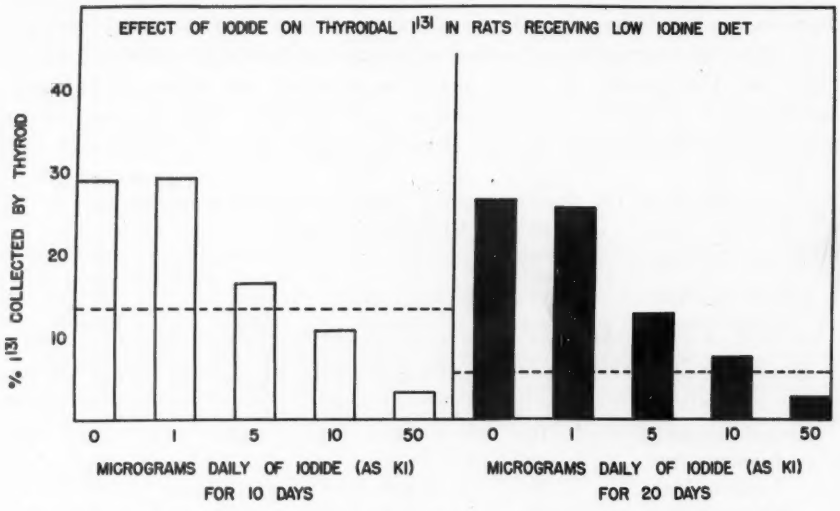

FIG. 1. The effect of daily administration of various amounts of iodide (as KI) on thyroidal collection of I^{131} in rats receiving low iodine diet.

generally between 10 and 16 per cent of the dose, but occasionally less than 10 per cent. The explanation for this is unknown. At twenty days, the control animals on the low iodine diet concentrated 26.7 per cent of the tracer dose of I^{131} in the thyroid. Although a dose of 1 microgram of KI was ineffective in decreasing this uptake, higher doses of KI did depress it.

Experiment V: The effect of low iodine intake on various indices of thyroid function

Animals were maintained on the low iodine diet for various periods of time. Periodically groups of rats (usually 10) were killed and thyroid function determined. The results obtained in this study are shown in Table 5 and Figure 2. It can be seen from Table 5 that the diet used in the study

TABLE 5. THE EFFECT OF LOW IODINE DIET ON THYROID FUNCTION

Duration of diet treatment (in days)	Body wt. (Gm.)	Thyroid wt. (mg.)	$\frac{\%\ I^{131}}{\text{Thyroid}}$	Total thyroid iodine (γ)	Serum	
					PBI (γ%)	Total iodine (γ%)
Purina diet	325	18.7	15.54	26.70	1.50	4.55
Low iodine diet:						
10 days	314	16.4	38.56†	21.54	1.96	2.93
20 days	307	18.3	46.58†	16.35	1.73	2.10
34 days	310	17.5	45.78†	12.74	1.73	1.76
106 days	279	25.3†	36.40†	5.30	0.76	1.10
237 days	337	55.5†	40.50†	2.75	0.90	2.05

† *See* footnotes to Table 1.

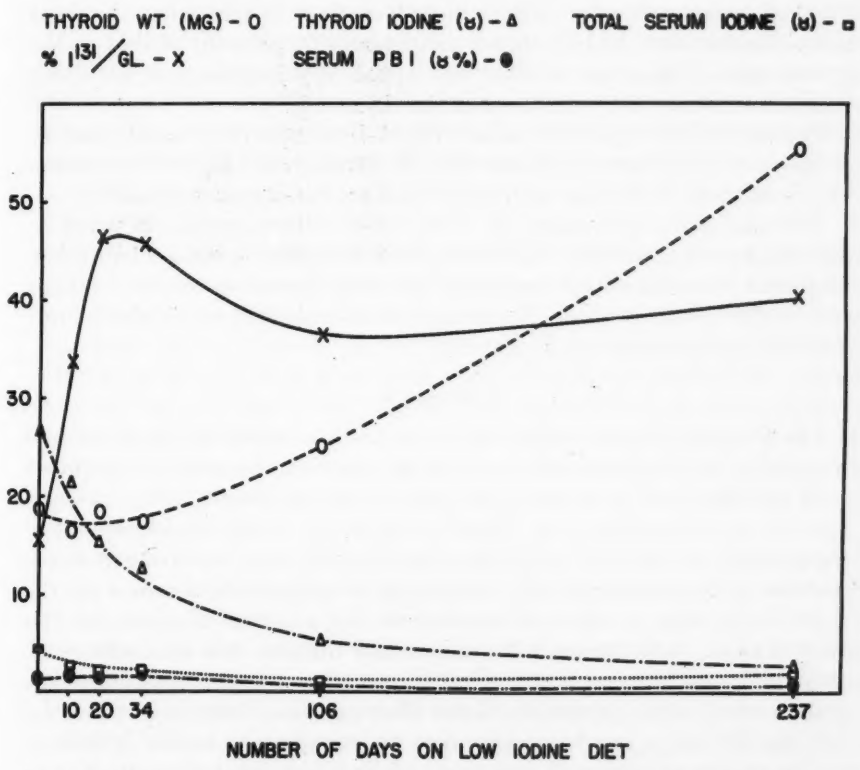

FIG. 2. The effect of low iodine diet on various indices of thyroid function in the rat. (Numbers on left are values for the various determinations listed at top of chart.)

did not appreciably depress body weight over fairly long periods of time. We have observed, however, that this diet does slow the growth rate of young actively growing animals.

That this diet probably did not contain a positive goitrogen is shown by the fact that thyroid weight increased slowly; even after 237 days of treatment it was only about three times greater than that in control rats. Animals maintained for ten months failed to show a greater increase in thyroid weight, although weights of 75 mg. were noted. This is far less than the weights which would be produced in animals receiving thiouracil for the same period of time.

The collection of I^{131} by the thyroid gland was rapidly increased during ten days on the diet. The maximum uptake seems to have occurred after about twenty days of treatment. From then on, there appeared to be a slight decrease in the collection.

The total thyroid iodine concentration did not fall markedly in the early stage of iodine deficiency. After thirty-four days of treatment the total thyroid iodine level had reached a value about 50 per cent of that in the control rats. This point of time was apparently beyond that at which maximum uptake of I^{131} occurred in the thyroid.

The serum PBI (protein-bound iodine) level was remarkably slow to change under treatment with this diet. By 106 days it had, however, fallen to a level about half of normal. From then on, it remains depressed.

The total iodine concentration in the blood fell rather rapidly when iodine intake was restricted. Within ten days, a marked reduction had taken place and by twenty days the value had been decreased to about 50 per cent of that in the controls. From then on, it continued to fall slowly, but probably not as much as did the PBI.

DISCUSSION

The foregoing studies clearly show that the low iodine diet we have used is suitable for investigations on thyroid function. Long-term treatment with this diet does produce an increase in thyroid weight. This increase, however, would appear to be related to decreased iodine intake and not to the presence of a positive goitrogen. Since most known positive goitrogens interfere in some manner with the uptake or utilization of iodine by the thyroid and since at all times throughout the administration of the diet there was no interference with radioiodine uptake, one can only reach the conclusion that no positive goitrogens were present. Therefore, the goiters which were eventually found after rats had been on the diet for 106 and 237 days, can be presumed to be secondary to iodine deficiency.

The studies of Levine, Remington and Von Kolnitz (1, 2) indicate that the diet they used contained about 15 micrograms of iodine per kilogram.

Our analysis of the diet we have used shows values for iodine which are about three times greater than their values (45.0 micrograms per kilogram of food—average of six determinations). Analysis of city tap water showed that our animals were probably getting between 0.1 and 0.2 microgram of iodine daily from this source. Food intake by our animals would indicate they were receiving between 1 and 2 micrograms of iodine per day. Therefore, the total intake of dietary iodine in our animals would appear to be in the neighborhood of 2 micrograms per day. Levine, Remington, and Von Kolnitz concluded that 3 micrograms of iodine as KI prevented the formation of a goiter. This amount probably is higher than the iodine intake of our animals. The discrepancy between their figures and ours might be explained by different methods of iodine analysis and different strains of animals.

In the experiment in which iodine was added to the intake of animals receiving the diet, no definite conclusion can be made as to the amount of iodine necessary for normal thyroid function in our strain of animals. The amount of iodine necessary to reverse the I^{131} collection to control values was approximately 5 to 10 micrograms per day. Concerning the facts that the control diet had a high iodine content and the serum inorganic iodine level was high, the I^{131} studies merely show that an intake of between 5 and 10 micrograms of iodine daily was necessary to obtain approximately the same serum inorganic iodine level as that obtained with the control diet.

These studies do not throw any additional light on the mechanism of goiter formation. However, it may be significant that thyroid weight does not increase markedly until the level of thyroid iodine has been decreased to about 50 per cent of the control level. This may suggest that rapid thyroid growth cannot take place as long as the thyroid iodine content remains high. It may further suggest that the gland becomes more sensitive to the growth-promoting effects of TSH at this time. This idea finds some support from the observations of Astwood and Bissell (8), who found that rapid thyroid growth resulted only when thiouracil had depleted thyroid iodine to low levels.

Without additional evidence it is difficult to determine the exact sequence of events which takes place when dietary iodine is low. From the present data it would appear that the most probable explanation for the occurrence of the changes seen in Figure 2 is as follows: The first two changes noted, *i.e.*, the increase in radioiodine collection by the thyroid and the decrease in the level of inorganic serum iodine, seemed to be causally related. To extrapolate from the uptake of a tracer dose of I^{131} to the actual amount of I^{127} that the thyroid accumulates, one must know the quantity of the pool of I^{127} that is labeled by the isotope. It is apparent

that if this pool is small, the thyroid may take up a large percentage of a label and a relatively small quantity of I^{127}. It would appear, then, that in all likelihood the increase in thyroidal collection of I^{131} in the first ten days of the experiment does not necessarily indicate any change in the thyroidal collection of I^{127} but is a function of the decreased concentration of I^{127} in the serum, or possibly in the thyroid. After a longer period of time on the iodine deficient diet, there is a gradual decrease in the thyroid iodine level, although the serum protein-bound iodine level remains constant. This would appear to indicate that the thyroid is secreting a normal amount of thyroid hormone at the expense of hormone stored in the gland. It is of considerable interest that the first point at which a change in the concentration of serum protein-bound iodine occurs, *i.e.*, 106 days, is also the first point at which an increase in thyroid weight is observed. This fits well with the hypothesis that a decrease in the level of circulating thyroid hormone causes an increase in the secretion of thyroid-stimulating hormone by the pituitary, the results of which are then observed as an increase in thyroid weight. As the diet is continued for 237 days, continued exposure of the pituitary to a decreased level of circulating thyroid hormone results in continued excessive secretion of thyroid-stimulating hormone and a further increase in thyroid weight. The apparent decrease in the collection of I^{131} by the thyroid beginning at 106 days may be related to a more rapid turnover of I^{131} by the thyroid. This is only conjecture at present, since no measurements of the serum PBI^{131} content were made.

The fact that the omission of any one component from the diet will change the amount of thyroidal I^{131} collection suggests that other factors besides iodine deficiency may be involved in the formation of an iodine-deficient goiter. Many possible substances have been suggested (9, 10) but none has been completely proven to be specific. Calcium has been implicated (11–14), and it is interesting to note that omission of this element from our diet produced the greatest decrease in thyroidal I^{131} collection. The exact meaning of this observation remains undetermined.

SUMMARY

1. An iodine-deficient diet has been used to determine experimentally some of the changes which occur in the rat thyroid when iodine intake is low.

2. Increased thyroidal collection of I^{131} appears to be associated with a decreased level of thyroid iodine and of serum inorganic iodine.

3. A fall in the level of serum PBI appears to be related to increased growth of the thyroid.

4. Short-term studies indicate that the effects of this diet may be reversed by administration of potassium iodide.

Acknowledgment

Valuable technical assistance during this study was furnished by Virginia Lucas, Jean Dorans, and Eveline Verace.

REFERENCES

1. LEVINE, H.; REMINGTON, R. E., and VON KOLNITZ, H.: Studies on the relation of diet to goiter. I. A dietary technic for the study of goiter in the rat, *J. Nutrition* **6:** 325, 1933.
2. LEVINE, H.; REMINGTON, R. E., and VON KOLNITZ H.: Studies on the relation of diet to goiter. II. The iodine requirement of the rat, *J. Nutrition* **6:** 347, 1933.
3. REMINGTON, R. E, and REMINGTON, J. W.: The effect of enhanced iodine intake on growth and on the thyroid glands of normal and goitrous rats, *J. Nutrition* **15:** 539, 1938.
4. ASTWOOD, E. B.: Action of antithyroid compounds, *Ann. New York Acad. Sci.* **50:** 419, 1949.
5. RAWSON, R. W., and MONEY, W. L.: Physiologic reactions of the thyroid stimulating hormone, *Recent Progr. Hormone Research* **4:** 397, 1949.
6. BARKER, S. B.: Determination of protein-bound iodine, *J. Biol. Chem.* **173:** 715, 1948.
7. NESH, F., and PEACOCK, W. C.: Notes on the use of radioactive iodine in protein bound iodine determinations, *Analyt. Chem.* **22:** 1573, 1950.
8. ASTWOOD, E. B., and BISSELL, A.: Effect of thiouracil on the iodine content of the thyroid gland, *Endocrinology* **34:** 282, 1944.
9. GREER, M. A.: Nutrition and goiter, *Physiol. Rev.* **30:** 513, 1950.
10. FERTMAN, M. B., and CURTIS, G. M.: Foods and the genesis of goiter, *J. Clin. Endocrinol.* **11:** 1361, 1951; also *Tr. Am. Goiter A.* p. 12, 1951.
11. THOMPSON, J.: Influence of the intake of calcium on the thyroid gland of the albino rat, *Anat. Rec.* **16:** 211, 1933.
12. WAHLBERG, J.: Deficiency of iodine and excess of calcium within an endemic goiter area, *Tr. Third Internat. Goiter Conf. and Am. A. Study Goiter* p. 71, 1938.
13. HELLWIG, C. A.: Experimental goiter due to calcium, *Arch. Surg.* **40:** 98, 1940.
14. SHARPLESS, G. R.; SABOL, M.; ANTHONY, E. K., and ARGETSINGER, H. L.: Goitrogenic action of calcium and vitamin D, *J. Nutrition* **25:** 119, 1943.
15. FISHER, R. A.: Statistical Methods for Research Workers, ed. 8. Edinburgh and London, Oliver and Boyd, Ltd., 1941.

DR. WILLIAM T. SALTER

WILLIAM THOMAS SALTER
1901–1952

WILLIAM THOMAS SALTER, physician, biochemist and pharmacologist, entered this world in Boston on December 19, 1901, and got his secondary schooling at that fine old academy, the Roxbury Latin School. In 1935 he married Eleanor Vallandingham, who survives him, together with three daughters.

From start to finish a good New Englander, Salter took his A. B. from Harvard in 1922 and his M.D. in 1925. That he made Phi Beta Kappa in college and Alpha Omega Alpha in medical school is evidence of the early excellence of his scholarship. That these achievements accurately presaged a distinguished career is confirmed by his receipt in 1941 both of an honorary A.M. from Yale, and more particularly of her chair of pharmacology with headship of that department.

Salter's medical internship and residency were at the Massachusetts General Hospital (1925-27 and 1927–28). After that he had a year in London as Moseley Travelling Fellow of Harvard (1928–29) working in the laboratory of Professor Charles R. Harington. On returning from this experience be continued to serve his alma mater in posts of increasing responsibility. For ten years (1929–39) he held the post of Tutor in Biochemical Sciences at Harvard College, a distinguished and unusual job for a doctor of medicine. During this same decade he rose from research fellow to Assistant Professor of Medicine in the Faculty of Medicine, and served as a member of the Cancer Commission of the University. Parallel hospital appointments included those of Associate Physician, first at the Huntington Hospital of Harvard, and then at the Thorndike Memorial of the Boston City Hospital. His final flowering, prematurely cut short by his untimely death, was in his last decade at Yale.

The writer first met "Bill" Salter at Woods Hole during his medical student days. It was quite evident even then that he had a deep interest in, and sound grasp of, biochemistry. However, he wanted to apply this fundamental discipine to clinical medicine, and that led to his hospital career. Salter's career exemplified indeed to an unusual degree the fusion of basic science and medical practice.

His exposure to Harington, the synthesizer of thyroxine, set the direction and pattern of his investigative career. Throughout the remainder of his life he was concerned with the thyroid hormone, its structure, its biosynthesis and metabolism, and the relation of the structure of its molecule to its physiologic activity. He made expert use of the new methods as they

came along, radioactive iodine, chromatography, and all the biochemical and biological methods applicable to his field.

His investigative work was reported, of course, steadily as he went along at scientific meetings and in scientific journals, but it was brilliantly assembled and integrated in his monograph published in 1940 entitled, "The Endocrine Function of Iodine." He was planning shortly before his death to bring this work up to date in the form of a second edition.

He received in April 1949 the Iodine Award for 1948 of the American Pharmaceutical Association—"For outstanding research in the pharmacy and chemistry of Iodine including fundamental pharmacological and clinical investigations relating to the function of iodine in nutrition and its role in metabolism."

Salter was not only a born investigator, but also a willing and inspiring teacher. The effectiveness of his teaching was enhanced by a puckish sense of humor. As was the Iodine monograph an expression of his love of research, so was the massive textbook of pharmacology which appeared just before his death an earnest of his devotion to education. Who can doubt that the vast labor which went into the preparation of this volume was contributory to his death.

For his drolleries as well as for his flashes of genius, but above all for his capacity for friendship, one loved Bill Salter. To visit his laboratory was a refreshing intellectual bath. To go through Woods Hole with him at the height of the tidal current in his tiny out-board motor boat (he knew nothing of boats) was high adventure. It was always great fun to share a platform with him; always there would be sprightly passages relieving the prosey presentation of mere science. One of his brightest gems was after a colleague had drawn some endocrinological conclusions to which he could not subscribe; he remarked that it is easy to prove that a monkey is faster than a greyhound if you hold the race through the tree tops!

Therefore, not only do we mourn a gifted contributor to our science and art, but a personality outstanding for its warmth and vigor. The memory of Bill will remain bright in the hearts of his friends. Medicine is the richer for his accomplishments but the poorer for loss of what more he might have accomplished had he been vouchsafed more years.

J. H. MEANS

The Endocrine Society

THE 1953 ANNUAL MEETING

The Thirty-fifth Annual Meeting of The Endocrine Society will be held in the Hotel Statler, New York, N. Y., on Thursday, Friday, and Saturday, May 28, 29 and 30, 1953.

The Committee on Local Arrangements is Dr. Earl T. Engle, Chairman, with Drs. Rulon W. Rawson and Sidney C. Werner as members of the Committee.

All Scientific Sessions will be held in the Hotel Statler. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Reservations should be made directly with the Hotel Statler, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send FOUR COPIES of the title and abstract to the Vice-President, Dr. Lawson Wilkins, Johns Hopkins Hospital, Baltimore 5, Maryland, not later than FEBRUARY 1, 1953. **It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:**

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three additional copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

The Endocrine Society

1953 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1953 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1954.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson in 1950. Dr. John D. Stoekle was chosen as the recipient of the 1951 Fellowship. Inasmuch as he was unable to accept, due to a call to military service, the same Fellowship was reawarded in 1952 to Dr. C. L. Courtright. The 1953 Schering Fellowship will be in the amount of $5,000 and will henceforth be given in the same amount on alternate years.

Awards

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH AWARD

This Award of $1,000 was established in 1939 by The Squibb Institute for Medical Research and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. None was given in 1943. This Award was increased to the amount of $2,500 beginning with the 1952 Award and the recipient was Dr. James H. Means. A special committee of five members of the Society selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1953.*

The American Goiter Association

1953 ANNUAL MEETING

The 1953 Annual Meeting will be held *May 7, 8 and 9, 1953,* in the Drake Hotel, Chicago, Illinois.

VAN METER PRIZE AWARD FOR 1953

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association, which will be held in Chicago, Illinois, May 7, 8 and 9, 1953, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, not later than *February 15, 1953.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association.